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P.2.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No. 38005-0121

*In re* patent application of  
KONTERMANN, Robert et al.

Serial No. US 09/288,719

Group Art Unit: 1632

Filed: April 09, 1999

Examiner: A. Beckerleg

For: SINGLE-CHAIN MULTIPLE ANTIGEN-BINDING MOLECULE, ITS  
PREPARATION AND USE

**DECLARATION UNDER 37 C.F.R. § 1.132**

Assistant Commissioner for Patents  
Washington D.C. 20231  
BOX AF

Sir:

**DECLARATION OF DR. ROLAND KONTERMANN**

I, Roland Kontermann, do declare as follows:

1. THAT, I am co-inventor of the invention of U.S. patent application serial No. 09/288,719 ("the application"). My curriculum vitae is provided as EXHIBIT (A) hereto.
2. THAT, I received a doctorate degree from the University of Heidelberg, Germany. I have worked in the field of protein engineering since 1992.

3. THAT, I was a research associate at the MRC centre for Protein Engineering in Cambridge, UK from 1993 to 1996.
4. THAT, I have been working in the field of targeting technologies for drug delivery for different diseases (such as, e.g. cancer) since 1993. I have published 14 papers, presented 20 abstracts and held 13 oral presentations at scientific meetings in the field of protein engineering. I published 5 papers and 18 meeting abstracts and oral presentations on engineered proteins for tumor therapy. My list of publications is provided as EXHIBIT (B) hereto.
5. THAT I have reviewed the Office Action of October 10, 2001 in the application. It is my understanding that the Examiner believes that the application fails to provide description for making of single chain binding molecules which bind to both a cell surface target molecule and a vector (page 4). According to the Examiner, the specification fails to guide one of skill to routes and methods of administration of a vector and single chain binding molecule such that a therapeutic effect on a target cell is observed (page 4)
6. THAT, I conducted and/or supervised experiments which employ the single chain multiple antigen-binding molecule disclosed and claimed in the subject application, used for efficient transfer of vectors into targeted cells. This system provides an effective means for gene therapy.
7. THAT, a person of ordinary skill in the art at the time the invention was filed was able to recognize based on the specification and the examples that the single-chain multiple antigen-binding molecules according to the invention are a versatile tool for in vitro and in vivo transfer of gene constructs into target cells, e.g. single-chain multiple antigen-binding molecules with a gene-construct specific ligand and a target cell specific ligand. The specification, the examples and the technologic knowledge of a skilled worker further more provide sufficient guidance to enable the skilled worker to con-

struct such single-chain multiple antigen-binding molecules according to the invention.

8. THAT, based on Example 1 of the application and the guidance provided in the specification and known teachings in the art, one of ordinary skill in the art could, by conventional means, construct other single chain multiple antigen binding molecules according to the claimed invention, *e.g.*, those which have a specificity for both a plasmid or viral component and a cell surface target molecule: Example 1 shows a single chain multiple antigen binding molecule that is capable of binding CEA and beta-galactosidase, comprising VH1-anti CEA, VL2-anti-beta-galactosidase, VH2-anti-beta-galactosidase, and VL1-anti-CEA (see specification at p. 27, l. 23 through p. 29, l. 31; see also figure 1). At the time of the invention a skilled artisan would have been able to replace the VH and VL specificities with those for a cell membrane of a target cell and a vector, respectively. Two methods conventionally used to clone VH and VL domains at the time the application was filed are: 1) recruiting established hybridoma-secreting antibodies using the desired specificity as the starting point; and. 2. use antibody phage display libraries to isolate specific antibody fragments (*e.g.*, ScFv, Fab). According to the first method, RNA is isolated from the hybridoma and is reverse transcribed into cDNA, using oligo-DT or random hexamer primers. The VH- and VL-encoding DNA is amplified, *e.g.*, by PCR, using a primer specific for the variable domains, as described by Dubel *et al.*, J. Immunol. Meth. 175:89-95 (1994), and Krebber *et al.*, J. Immunol. Meth. 201:35-55 (1997). According to the second method, such phage display libraries are either generated from immunized (*i.e.*, immune library) or non-immunized (*i.e.*, naiv library) donors, or are generated synthetically by a genetic engineering, as described by Winter *et al.*, Annu. Rev. Immunol. 12:433-55 (1994), and Vaughan *et al.*, Nature Biotechnol. 14:309-14 (1996). Then, specific antibodies are selected using, for example, immobilized purified polypeptides (Nissim *et al.*, J. Immunol. Meth. EMBO J. 13:692-98 (1994)), whole cells (De Kruif *et al.*, J. Mol. Biol. 248:97-105 (1995)), or tissue sections

(Tordsson *et al.*, J. Immunol. Meth. 210:11-23 (1997)). Each method has been successfully applied in various contexts. For example, antibodies have been isolated against DNA, as shown by Young *et al.*, Mol. Immunol. 35:1207-17 (1998). Antibodies have been isolated against viral code proteins as disclosed by Watkins *et al.*, Gene Ther. 4:1004-12 (1996). Also antibodies have been isolated against a large panel of different cell surface antigens such as CEA (Osborne *et al.*, Immunotech. 2:181-96 (1996)) and HMW-MAA (Noronha *et al.*, J. Immunol. 161:296876 (1998)).

As described in the following detailed protocol, the DNA encoding the VH and VL fragments is amplified by PCR with the indicated primers which anneal in the 5' or 3' region, respectively, of the variable regions and which introduce appropriate cloning sites. These fragments are combined into a single-chain multivalent antigen-binding protein by standard molecular techniques generally known to the person skilled in the art. These cloning steps follow protocols published earlier (Kontermann *et al.*, 1997, Immunotechnology 3, 137-144; Kontermann *et al.*, Nature Biotechnology 15, 629-632) and are generally known to the person skilled in the art.

Bispecific single-chain multivalent antigen-binding proteins are generated by converting the first antigen-binding molecule into a bivalent diabody molecule (Holliger *et al.*, 1993, Proc. Natl. Acad. Sci. USA 90, 6444-6448). The variable domains of the second specificity are then inserted into this bivalent diabody construct to obtain a bispecific single-chain multivalent antigen-binding protein. Due to the strong conservation of the 5' and 3' regions of the variable light and heavy chain genes, basically any antibody molecules can be converted to a bispecific single-chain multivalent antigen-binding proteins by means of standard molecular biology techniques (Sambrook *et al.*, 1989, Molecular Cloning, Cold Spring Harbor Laboratory). Bivalent diabodies are generated by substituting the original linker between the VH and VL domain of a single-chain Fv Fragment by a short peptide linker. The standard linker, which is also used in this protocol, is five amino

acids long (with the sequence -GGGGS-). Two configurations are possible: the  $V_H$ - $V_L$  configuration, fusing the  $V_L$  domain to the C-terminal of the  $V_H$  domain, and the  $V_L$ - $V_H$  configuration, with the  $V_H$  domain fused to the C-terminal of the  $V_L$  domain. The cloning strategy is based on the introduction of appropriate cloning sites into the  $V_H$  and  $V_L$  fragments. For the construction of bispecific molecules in the HL configuration, which is described here, a Bst EII site is introduced at the 3' region of the  $V_H$  fragment (if not already present) and a Sac I site into the 5' region of the  $V_L$  fragment. Bispecific molecules are then generated by combining the  $V_H$  and  $V_L$  fragments from two different antibodies. For this purpose an AscI site is used to combine the DNA fragments. The  $V_H$  and  $V_L$  fragments of an antibody with a second specificity are amplified by PCR to introduce appropriate cloning sites and the middle linker. These fragments are then cloned into a plasmid containing a bivalent diabody in the same configuration. Due to the presence of the middle linker, with a length of 15 amino acids, it is not necessary to introduce a second ribosome binding site and an additional leader sequence.

#### *Restriction site analysis*

Restriction sites used for construction in the sequence of the  $V_H$  and  $V_L$  fragments of the antibody fragments to be convert into a bispecific single-chain multivalent antigen-binding proteins are checked for. If additional sites are found partial digests or multiple fragment ligation might have to be used for the generation of antibody fragments. Alternatively, these sites can be deleted by site-directed mutagenesis. It is also possible to introduce other restriction sites suitable for cloning. We have found that most antibody fragments can be cloned as diabodies or single-chain diabodies using the above described restriction sites.

#### *Generation of bivalent diabodies in the $VH$ - $VL$ configuration*

Bivalent diabodies are generated by linking a  $V_H$  and a  $V_L$  domain obtained from the same antibody with a short interdomain linker. An antibody fragment (e.g. a scFv, or Fab fragment) cloned into a bacterial pUC19-derived

expression vector such as pAB1 (Kontermann et al., 1997, Immunotechnology 3, 137-144) can be used as starting material for the generation of a bivalent diabody.

1. Oligonucleotides for amplification of the VH and VL fragments are designed. Approximately 20-30 nucleotides derived from your antibody sequence are used for annealing.
2. Primer LMB3 is used for the VH fragment (annealing in the vector backbone) and a forward primer VHABstFor (introducing a Bst EII site in the 3' region of the VH fragment).
3. Primer LMB2 is used for the VL fragment (annealing in the vector backbone) or fdSeq1 (if the antibody fragment is fused to g3p, i.e. isolated from a phage library) and primer VLABstSacBack annealing in the 5' region of the VL fragment and a Bst EII site, the 5 amino acid linker sequence, and a Sac I site are added to the VL fragment.
4. The VH and the VL fragments are amplified with the respective primers by PCR. Different polymerases including proof-reading ones are used. We routinely perform 25 cycles with an annealing temperature of 50 or 55°C.
5. PCR products are Gel purified. The two fragments should run at approximately 350 bp.
6. The amplified VH fragment is digested with SfiI and Bst EII and the VL fragment with Bst EII and Not I. The reaction conditions supplied by the manufacturer are used. Note: Sfi I needs 50°C reaction temperature and Bst EII 60°C.
7. A bacterial expression vector, such as pAB1, is digested which contains a pelB leader sequence and Sfi I and Not I sites in the multiple cloning site. The digested vector is dephosphorylated with calf intestine alkaline phosphatase.
8. The digested fragments and vector are purified by standard protocols (e.g. phenol/chloroform extraction and ethanol precipitation or using commer-

cially available spin-columns). The amounts are estimated by running aliquots on a 1% agarose gel.

9. The VH and VL fragments are ligated with the vector fragment at 15°C overnight using VH:VL:vector ratios of approximately 2:2:1 in a total volume of 20 µl.
10. 10 µl of the ligation reaction are transformed into TG1 competent cells using standard protocols and plate cells onto TYE, 100 µg/ml ampicillin, 1% glucose plates. Incubate overnight at 37°C.
11. Positive clones are screened by PCR with primers LMB2 and LMB3. To perform the screen, 12-24 single colonies are picked from the plate with sterile toothpicks, dipped into 20 µl of PCR reaction mix (e.g. aliquoted into a 96 well PCR plate) and then streaked it onto a TYE, 100 µg/ml ampicillin, 1% glucose plate (master plate). 30 cycles of PCR are run at an annealing temperature of 50°C and an extension time of 1 min. The PCR products are analyzed on a 1% agarose gel. Positive clones should give a product of approximately 900 bp (VH-VL insert plus flanking vector-derived sequences).
12. Positive clones are analyzed for expression of full-length antibody sequence by immunoblot experiments of bacterial pellets of induced overnight cultures with anti-Myc tag antibody 9E10. Alternatively, the supernatant of an induced 2 ml culture in ELISA is directly checked for antigen binding (protocol 6). The culture is first grown in 2xTY, 100 µg/ml ampicillin, 0.1% glucose until an OD600 of 0.8-1.0 is reached, then 1 mM IPTG are added and incubated at shaking conditions overnight at 30°C.

#### *Generation of bispecific diabodies in the VH-VL configuration*

1. The oligonucleotides are designed for amplification of the VH and VL fragments of the second antibody. Approximately 20-30 nucleotides derived from your antibody sequence are used for annealing.
2. Primers VHBAscBack (annealing in the leader sequence and adding an AscI site and the second half of the middle linker sequence) and

- VHBSacFor (annealing at the 3' region of the VH fragment and adding a 5 amino acid linker and a SacI site) are used for the VH fragment.
3. Primers VLBBstBack (annealing in the 5' end of the VL fragment and adding a Bst EII site and 5 amino acid linker) and VLBAscFor (annealing in the 3' end of the VL fragment and adding the first half of the middle linker sequence and an Asc I site) are used for the VL fragment.
  4. The VH and VL fragments with the respective primers are amplified by PCR and fragments are purified as described in protocol 2.
  5. The VH fragment are digested with AscI and Sac I and the VL fragment with Bst EII and Asc I.
  6. The bivalent diabody construct in the HL configuration is digested with Bst EII and Sac I.
  7. The VLB and VHB fragments is ligated into digested plasmid DNA as described above.
  8. The identification of positive clones proceeds as described above.

*Primers:*

LMB3 5'-CAG GAA ACA GCT ATG ACC-3'

LMB2 5'-GTA AAA CGA CGG CCA GT-3'

fdSeq1 5' GAA TTT TCT GTA TGA GG-3'

Primers for amplification of individual V<sub>H</sub> and V<sub>L</sub> domains (restriction sites are underlined, dots indicate bases which have to be derived from individual sequences, for annealing these regions should have a length of approximately 20 bases):

V<sub>H</sub>ABstFor: 5'-CGA GGA GAC GGT GAC CAG ... ...-3' (reverse primer which anneals in the 3' end of V<sub>L</sub>A and introducing a *Bst*E II site in the region encoding framework 4).

V<sub>L</sub>ABstSacBack: 5'-ATC CTG GTC ACC GTC TCC TCG GGC GGT GGC GGA TCC GAT ATC GAG CTC ... ...-3' (anneals in the 5' end of V<sub>L</sub>A and introduces a *Bst*E II site, the GGGGS-linker encoding region and a *Sac* I site into V<sub>L</sub>A).



V<sub>H</sub>BSacFor: 5'-TCG GAG CTC GAT GTC CGA TCC GCC CAA GCC ...  
...-3' (reverse primer which anneals in the 3' end of V<sub>H</sub>B and introduces  
DNA encoding a 5 amino acid GGGS linker and a Sac I site).

V<sub>L</sub>BBstBack: 5'-GAT CTG GTC ACC GTC TCC TCA GGC GGT GGC  
GGA TCG ... ...-3' (anneals in the 5' end of V<sub>L</sub>B and introduces a *BstE* II  
site)..

V<sub>H</sub>BAscBack: 5'- TAA GGG CGC GCC TCG GCT GGT AAT ACT AGT  
... ...-3' (anneals in the 5' end of V<sub>H</sub>B and introduces an *Asc I* site followed  
by DNA encoding for the second part of middle linker M)

V<sub>L</sub>BAscFor: 5'- GCA GGC GCG CCC AGC ATT ACT ATC ACT ACC ...  
...-3' (reverse primer which anneals in the 3' end of V<sub>L</sub>B and introduces  
DNA encoding for the first part of middle linker M followed by an *Asc I*  
site)

9. THAT, the fusogenic peptides listed in EXHIBIT (C) are the same fusogenic peptides disclosed in DE 196 49 645.4, page 10, lines 30-64 and incorporated by reference into the application at page 10, lines 10 to 14.
10. THAT, the target cell specific ligands listed in EXHIBIT (D) are the same ligands disclosed in DE 196 49 645.4, page 3, line 46 through page 9, line 63 and incorporated by reference into the referring application US 09/288,719, page 10, lines 10-14.
11. THAT, based on the disclosure of the target cell specific ligands disclosed in DE 196 49 645.4, page 3, line 46 through page 9, line 63 and incorporated by reference into the application at page 10, lines 10-14 and further based on the specification of the application and the technical knowledge of one of ordinary skill in the art, such skilled artisan would have been able to identify and construct the V<sub>H</sub> and/or V<sub>L</sub> sequences useable as target cell specific ligands. Examples of sequences of such target cell specific ligands are listed in EXHIBIT (E).

12. THAT, gene construct-specific ligands listed in EXHIBIT (F) and incorporated by reference to DE 196 49 645 A1 (listed on page 11, line 55 through page 13, line 40) are the same as the gene construct-specific ligands cited in the application at page 10, lines 10-14..
13. THAT, based on the disclosure of the gene construct-specific ligands disclosed in DE 196 49 645.4, page 11, line 55 through page 13, line 40 and incorporated by reference into the application U at page 10, lines 10-14 and further based on the specification of the application and the technical knowledge one of ordinary skill in the art, such skilled artisan would have been able to identify and construct the VH and/or VL sequences useable as gene construct-specific ligands. Examples of sequences of such gene construct-specific ligands are listed in EXHIBIT (G).
14. THAT, a person ordinarily skilled in the art was able to identify and construct the VH and/or VL sequences useable as gene construct-specific ligands based on the specification of the application (p. 27, lines 1 to 3: "*Examples of antibodies against viral antigens are: anti-HBV, anti-HCV, anti-HPV, anti-HTLV, anti-coxsackievirus or, anti-hantavirus.*"), example 1 and the technical knowledge of the skilled artisan. Such VH or VL sequences are listed in EXHIBIT (G).
15. THAT, a person skilled in the art at the time the invention was filed, based on the information provided in the specification and the knowledge at the time the invention was able to construct single chain multiple antigen binding molecules which bind to both a cell surface target molecule and a vector. Examples of sequences of single chain multiple antigen binding molecules comprising
- a gene construct-specific ligand selected from the list in EXHIBIT (F) and EXHIBIT (G),
  - a target cell-specific ligand selected from the list in EXHIBIT (D) and EXHIBIT (E)

are described in EXHIBIT (H)

16. THAT, I supervised and/or conducted experiments based on the examples and the specification of the application that prove that based on the information provided in the specification and the knowledge at the time the invention was filed an ordinarily skilled person in the art was able to construct single chain multiple antigen binding molecules which bind to both a cell surface target molecule (human endoglin CD 105) and a vector (adenoviral envelope protein: adenovirus fiber knob domain scDb EDG-Ad) as published by my team Nettelbeck et al., Molec. Ther. 3 (6):882-91 (2001)): The single chain multiple antigen binding molecule described therein is able to bind a vector (adenovirus) and a cell (endothelial cell expressing endoglin CD 105), This further demonstrates the utility of the single chain multiple antigen binding molecules according to the invention for targeting of adenovirus containing a gene construct, preferably a genetherapeutically active gene construct, to cells such as for example cancerous cells.
17. THAT, all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with knowledge that willful false statements and the like so are made punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing hereon.

Date: 15 Feb. 2002 Signature: R. Kontermann

#### EXHIBITS

EXHIBIT (A): Curriculum vitae of Dr. R. Kontermann

EXHIBIT (B): List of publications of Dr. R. Kontermann

EXHIBIT (C): Fusogenic peptides cited in DE 196 49 645.4

EXHIBIT (D): Target cell specific ligands cited in DE 196 49 645.4

EXHIBIT (E): Target cell specific ligands

EXHIBIT (F): Gene construct-specific ligands cited in DE 196 49 645.4

EXHIBIT (G): Gene construct-specific ligands

EXHIBIT (H): Sequences of single chain multiple antigen binding molecules

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**EXHIBIT A**

**Curriculum vitae**

*Roland E. Kontermann, Ph.D.*

*- Molecular Biologist -*

Address	Lerchengasse 5, 35085 Ebsdorfergrund, Germany
Date of birth	6 February 1961
Place of birth	Urbach (Germany)
Nationality	German
Marital status	married

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since Jan. 2001	Head of the Biotechnology Department of vectron therapeutics AG
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2001	Habilitation (venia legendi) in Molecular Biology
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March 1996 - Dec. 2000	research associate at the Institute of Molecular Biology and Tumor Research of the University of Marburg
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Oct. 1993 neering - Feb. 1996	research associate at the MRC Centre for Protein Engi- (Cambridge, England) in the laboratory of Dr. Greg Winter
July 1992 - Sept. 1993	research associate at the Institute of Molecular Genetics of the University of Heidelberg
July 1992	Ph.D. in molecular biology
Jan. 1989 - July 1992	Ph.D. thesis at the Institute of Molecular Genetics of the University of Heidelberg
Aug. 1987 sity of - Nov. 1988	civil service at the Institute of Immunology of the Univer- Heidelberg
Sept. 1987	Diploma in biology
1986 - 1987 University	Diploma thesis at the Institute of Molecular Genetics of the of Heidelberg
1980 - 1987	studied biology at the University of Hohenheim subjects: genetics, zoology, biochemistry, microbiology
1977 - 1980	grammar school of economy
1971 - 1977	intermediate school
1967 - 1971	elementary school

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**EXHIBIT B**

**List of Publications of Roland E. Kontermann**

**A. Scientific publications**

1. **Kontermann, R., Sitzler, S., Seifarth, W., Petersen, G. & Bautz, E.K.F. (1989)**  
Primary structure and functional aspects of the gene coding for the second-largest subunit of RNA polymerase III of *Drosophila melanogaster*.  
*Mol. Gen. Genet.* **219**, 373-380.
2. **Kontermann, R. & Rauterberg, E.W. (1989)**  
N-deglycosylation of human complement component C9 reduces its hemolytic activity.  
*Mol. Immunol.* **26**, 1125-1132.
3. **Kontermann, R., Deppisch, R. & Rauterberg, E.W. (1990)**  
Several epitopes of human complement C9 are involved in interaction with the C5b-8 complex and other C9 molecules.  
*Eur. J. Immunol.* **20**, 623-628.
4. **Seifarth, W., Petersen, G., Kontermann, R., Riva, M., Huet, J. & Bautz, E.K.F. (1991)**

Identification of the genes for the second-largest subunits of RNA polymerase I and III of *Drosophila melanogaster*.

*Mol. Gen. Genet.* **228**, 424-432.

5. Ng, S.W., Wiedemann, M., **Kontermann, R.** & Petersen, G. (1992)  
Molecular characterization of a putative peroxidase gene of *Drosophila melanogaster*.  
*Biochim. Biophys. Acta* **1171**, 224-228.
6. **Kontermann, R.** & Bautz, E.K.F. (1992)  
Similarity between subunit 8 of yeast RNA polymerase II (RPB8) and the second-largest subunits of eukaryotic RNA polymerases.  
*Nucleic Acids Res.* **20**, 5321.
7. Liu, Z., **Kontermann, R.E.**, Schulze, R.A., Petersen, G. and Bautz, E.K.F. (1993)  
*RP115* codes for the M<sub>r</sub> 15 000 subunit 9 of *Drosophila melanogaster* RNA polymerase II.  
*FEBS Lett.* **335**, 73-75.
8. **Kontermann, R.E.**, Kobor, M. & Bautz, E.K.F. (1993)  
Identification of a nucleic acid-binding region within the largest subunit of *Drosophila melanogaster* RNA polymerase II.  
*Protein Sci.* **2**, 223-230.
9. Schulze, R.A., **Kontermann, R.E.**, Queitsch, I., Dübel, S. & Bautz, E.K.F. (1994)  
Thiophilic adsorption chromatography of recombinant single-chain antibody fragments.  
*Anal. Biochem.* **220**, 212-214.
10. **Kontermann, R.E.** & Bautz, E.K.F. (1994)  
Nucleic acid-binding regions of the second-largest subunit of *Drosophila* RNA polymerase II identified by Southwestern blotting.  
*FEBS Lett.* **334**, 166-170.
11. Griffiths, A.D., Williams, S.C., Hartley, O., Tomlinson, I.M., Waterhouse, P., Crosby, W.L., **Kontermann, R.E.**, Jones, P.T., Low, N.M., Allison, J., Prospero, T.D., Hoogenboom, H.R., Nissim, A., Cox, J.P.L., Harrison, J.L., Zaccolo, M., Gherardi, E. & Winter, G. (1994)



Isolation of high affinity human antibodies directly from large synthetic repertoires.

*EMBO J.* **13**, 3245-3260.

12. Kipriyanov, S.M., Dübel, S., Breitling, F., **Kontermann, R.E.** & Little, M. (1994)  
Recombinant single-chain Fv fragments carrying C-terminal cysteine residues: production of bivalent and biotinylated miniantibodies.  
*Mol. Immunol.* **31**, 1047-1058.
13. Dübel, S., Breitling, F., **Kontermann, R.**, Schmidt, T., Skerra, A. & Little, M. (1995)  
Bifunctional and multimeric complexes of streptavidin fused to single chain antibodies (scFv).  
*J. Immunol. Meth.* **178**, 201-209.
14. Kipriyanov, S.M., Dübel, S., Breitling, F., **Kontermann, R.E.**, Heymann, S. & Little, M. (1995)  
Bacterial expression and refolding of single-chain Fv fragments with C-terminal cysteines.  
*Cell Biophysics* **26**, 187-204.
15. **Kontermann, R.E.**, Liu, Z., Schulze, R.A., Sommer, K.A., Queitsch, I., Dübel, S., Kipriyanov, S.M., Breitling, F. & Bautz, E.K.F. (1995)  
Characterization of the epitope recognized by a monoclonal antibody directed against the largest subunit of *Drosophila* RNA polymerase II.  
*Biol. Chem.* **376**, 473-481.
16. Fisch, I., **Kontermann, R.E.**, Finnern, R., Hartley, O., Soler-Gonzales, A., Griffiths, A.D. & Winter, G. (1996)  
A strategy of exon shuffling for making large peptide repertoires displayed on filamentous bacteriophage.  
*Proc. Natl. Acad. Sci. USA* **93**, 7761-7766.
17. **Kontermann, R.E.**, Martineau, P., Cummings, C.E., Karpas, A., Allen, D., Derbyshire, E. & Winter, G. (1997)  
Enzyme immunoassays using bispecific diabodies.  
*Immunotechnology* **3**, 137-144.
18. **Kontermann, R.E.**, Wing, M.G. & Winter, G. (1997)  
Complement recruitment using bispecific diabodies.

*Nature Biotechnology* **15**, 629-631.

19. Heidtmann, H.-H. & Kontermann, R.E. (1998)  
Cloning and recombinant expression of mouse coagulation factor X.  
*Thromb. Res.* **92**, 33-41.
20. Brüsselbach, S., Korn, T., Völkel, T., Müller, R. & Kontermann, R.E. (1999)  
Enzyme recruitment and tumor cell killing in vitro by a secreted bispecific single-chain diabody.  
*Tumor Targeting* **4**, 115-123.
21. Kontermann, R.E. & Müller, R. (1999)  
Intracellular and cell surface displayed single-chain diabodies.  
*J. Immunol. Meth.* **226**, 179-188.
22. Alt, M., Müller, R. & Kontermann, R.E. (1999)  
Novel tetravalent and bispecific IgG-like antibody molecules combining single-chain diabodies with the immunoglobulin  $\square$ 1 Fc or CH3 region.  
*FEBS Lett.* **454**, 90-94
23. Heidtmann, H.-H., Nettelbeck, D.M., Mingels, A., Jäger, R., Welker, H.-G. & Kontermann, R.E. (1999)  
Generation of angiostatin-like fragments from plasminogen by prostate-specific antigen.  
*Br. J. Cancer* **81**, 1269-1273.
24. Nettelbeck, D.M., Miller, D.W., Jérôme, V., Zuzarte, M., Watkins, S.J., Hawkins, R.E., Müller, R. & Kontermann, R.E. (2001)  
Targeting of adenovirus to endothelial cells by a bispecific single-chain diabody directed against the adenovirus fiber knob domain and human endoglin (CD105).  
*Mol. Ther.* **3**, 882-891.
25. Völkel, T., Korn, T., Bach, M., Müller, R. & Kontermann, R.E. (2001)  
Optimised linker sequences for the expression of monomeric and dimeric bispecific single-chain diabodies  
*Protein Eng.* **14**, 815-823.

**B. Reviews**

1. **Kontermann, R.E.** (2000)  
Recombinant antibody fragments for cancer therapy.  
Mod. Asp. Immunobiol. 1, 88-91.

**C. Books and book chapters**

1. **Kontermann, R.E. & Dübel, S.** (Hrsg.) (2001)  
Antibody engineering, a lab manual.  
Springer-Verlag Heidelberg.
2. **Dübel, S. & Kontermann, R.E.** (2001)  
Recombinant antibodies  
*In: Antibody engineering, a lab manual.* Kontermann, R.E. & Dübel, S. (Hrsg.)  
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3. **Kontermann, R.E.** (2001)  
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*In: Antibody engineering, a lab manual.* Kontermann, R.E. & Dübel, S. (Hrsg.)  
Springer-Verlag Heidelberg. pp 137-148.
4. **Winter, C.H. & Kontermann, R.E.** (2001)  
Epitope mapping with gene-fragment libraries.  
*In: Antibody engineering, a lab manual.* Kontermann, R.E. & Dübel, S. (Hrsg.)  
Springer-Verlag Heidelberg. pp 473-490.
5. **Korn, T., Völkel, T. & Kontermann, R.E.** (2001)  
Bivalent and bispecific diabodies and single-chain diabodies.  
*In: Antibody engineering, a lab manual.* Kontermann, R.E. & Dübel, S. (Hrsg.)  
Springer-Verlag Heidelberg. pp 619-636.
6. **Kontermann, R.E., Korn, T. & Jérôme, V.** (2002)  
Recombinant adenoviruses for *in vivo* expression of antibody fragments.

*In: Recombinant Antibody Technology for Cancer Therapy: Reviews and Protocols. Welschhof, M. & Krauss, J. (Hrsg.), Series "Methods in Molecular Medicine", Humana Press, in press.*

7. **Kontermann, R.E., (2002)**

Single-chain Fv fragments from phage display libraries.

*In: Vector Targeting for Therapeutic Gene Delivery. Curiel, D.T. & Douglas, J.T. (Hrsg.), Wiley & Son, in press.*

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Production of recombinant bispecific antibodies.

*In: Antibody Engineering Protocols, 2<sup>nd</sup> edition - Methods in Molecular Biology Vol. 51. Lo, B.K.C. (Hrsg.), Humana Press, USA, in preparation.*

**D. Abstracts and short oral presentations**

1. **Kontermann, R., Sitzler, S., Seifarth, W. & Bautz, E.K.F. (1988)**

DNA sequence analysis of RNA polymerase genes of *Drosophila*.

*20. Jahrestagung der Deutschen Gesellschaft für Genetik, Salzburg.*

2. **Bausback, J., Kontermann, R. & Rauterberg, E.W. (1988)**

Epitope mapping on complement C9 by monoclonal antibodies.

3. **Bausback, J., Kontermann, R. & Rauterberg, E.W. (1988)**

Functionally important domains of C9 as defined by monoclonal antibodies inhibiting hemolysis.

*Jahrestagung der Gesellschaft für Immunologie, Düsseldorf.*

4. **Kontermann, R. & Rauterberg, E.W. (1989)**

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*5. Frühjahrstagung der Gesellschaft für Immunologie, Freiburg.*

5. **Kontermann, R., Bausback, J. & Rauterberg, E.W. (1989)**

Polymerisation of C9 is no prerequisite for its lytic function.

*International Congress of Immunology, Berlin.*

6. Seifarth, W., Sitzler, S., **Kontermann, R.**, Petersen, G. & Bautz, E.K.F. (1989)  
Distribution of RNA polymerase II and III on polytene chromosomes of *Drosophila melanogaster*.  
*11. European Drosophila Research Conference, Marseille.*
  
7. Boenisch, S., **Kontermann, R.** & Rauterberg, E.W. (1990)  
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8. Ng, S.-W., **Kontermann, R.**, Petersen, G. & Bautz, E.K.F. (1990)  
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9. Ng, S.-W., Wiedemann, M., **Kontermann, R.** & Petersen, G. (1991)  
Molecular analysis of cDNA and genomic sequences encoding a putative peroxidase gene of *Drosophila melanogaster*.  
*12. European Drosophila Research Conference, Mainz.*
  
10. **Kontermann, R.** & Bautz, E.K.F. (1991)  
The DNA-binding domain of the largest subunit of RNA polymerase II of *Drosophila melanogaster*.  
*23. Jahrestagung der Gesellschaft für Genetik, Berlin.*
  
11. **Kontermann, R.** & Bautz, E.K.F. (1992)  
Nucleic acid-binding regions of the two large subunits of *Drosophila* RNA polymerase II.  
*Symposium on structural tools for the analysis of protein-nucleic acid complexes, Wildbad Kreuth.*
  
12. **Kontermann, R.**, Kobor, M. & Bautz, E.K.F. (1992)  
Regions of the two large subunits of *Drosophila* RNA polymerase II interacting with nucleic acids.  
*24. Jahrestagung der Gesellschaft für Genetik, Tübingen.*
  
13. **Kontermann, R.E.**, Williams, S.C., Hartley, O., Tomlinson, T., Waterhouse, P., Crosby, W.L., Jones, P.T., Low, N.M., Allison, J., Prospero, T.D., Hoogenboom, H.R., Nissim, A., Cox, J.P.L., Harrison, J.L., Zaccolo, M., Gherardi, E., Winter, G. & Griffiths, A.D. (1994)

Isolation of high affinity antibodies directly from large phage-display antibody repertoires.

*10. Frühjahrstagung der Gesellschaft für Immunologie, Heidelberg.*

14. **Kontermann, R.E.,** Wing, M.G. & Winter, G. (1996)

Complement recruitment using bispecific diabodies.

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Complement recruitment using bispecific diabodies.

*Antibody Technology and Applications in Health and Environment (GBF-Symposium), Braunschweig.*

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Complement recruitment using bispecific diabodies.

*IBC Antibody Engineering Conference, San Diego, USA.*

17. **Kontermann, R.E.,** Alt, M., Korn, T., Völkel, T., Müller, R. & Brüsselbach, S. (1999)

Bispecific single-chain diabodies.

*15. Frühjahrstagung der Gesellschaft für Immunologie, Stuttgart.*

18. Montigiani, S., Müller, R. & **Kontermann, R.E.** (1999)

Inhibition of cell proliferation by E2F-specific peptides interfering with DNA binding.

*Cell Cycle Conference, Lausanne.*

19. Nettelbeck, D.M., Watkins, S.J., Hawkins, R.E., Müller, R. & **Kontermann, R.E.** (1999)

Adenovirus targeting to tumor endothelial cells using bispecific multivalent ligands (MVL) consisting of an anti-knob scFv fused to VEGF.

*Cold Spring Harbor Laboratory Meeting on 'Vector targeting Strategies for Therapeutic Gene Delivery'.*

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Engineering of factor X variants activated by prostate-specific antigen.

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Bispecific single-chain diabodies.  
*VI<sup>th</sup> International Conference on Bispecific Antibodies and Related Strategies for Targeted Immune Modulation, Monterey, USA.*
  
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25. Kontermann, R.E., Korn, T., Völkel, T., Alt, M., Müller, R. & Brüsselbach, S. (1999)  
Bispecific single-chain diabodies secreted from mammalian producer cells for the recruitment of effector mechanisms to tumour cells.  
*VII<sup>th</sup> Meeting of the European Society of Gene Therapy, München. Veröffentlicht in J. Gene Med. 1 Suppl., 48.*
  
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*First International Symposium on Gene Technology & Skin Gene Therapy. Essen.*

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Bispecific single-chain diabodies.

*Biotechnology 2000, Berlin*

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Bispecific single-chain diabodies for tumour targeting.

*International Society for Oncodevelopmental Biology and Medicine - ISOBM Meeting, München.*

31. **Kontermann, R.E.**, Nettelbeck, D.M., Miller, D.W., Watkins, S.J., Hawkins, R.E., Müller, R. (2000)

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*European Society of Gene Therapy - ESGT, Stockholm.*

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Linkermania: Connecting variable antibody domains to form multivalent molecules.

*11<sup>th</sup> IBC Antibody Engineering Conference, La Jolla, USA.*

33. Völkel, T., Heidtmann, H.-H., Alt, M., Müller, R. & **Kontermann, R.E.** (2001)

Engineering of factor X variants activated by prostate-specific antigen.

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34. Nettelbeck, D.M., Korn, T., Miller, D.W., Völkel, T., Watkins, S.J., Hawkins, R.E., Müller, R. & **Kontermann, R.E.** (2001)



Bispecific single-chain diabodies for the retargeting of vectors and effectors.  
*Cold Spring Harbor Laboratory Meeting on 'Vector Targeting Strategies for Therapeutic Gene Delivery'.*

#### E. Oral presentations

1. Isolation of high affinity antibody fragments directly from large antibody repertoires.  
*RNA Polymerase Symposium, Akademie der Wissenschaften, Heidelberg (Oktober 1994)*
2. Isolation of high affinity antibody fragments directly from large antibody repertoires.  
*MPI für Immunbiologie, Freiburg, i.B. (Februar 1995)*
3. Isolation of high affinity antibody fragments directly from large antibody repertoires.  
*Klinik für Tumorbologie, Freiburg, i.B. (Februar 1995)*
4. Bispezifische Diabodies für die Rekrutierung von Effektorfunktionen.  
*Behringwerke Marburg (1996)*
5. New developments in filamentous phage display technology – isolation of high affinity antibody fragments and peptides.  
*36. Biochemie-Kolloquium des Instituts für Biochemie der Friedrich-Schiller-Universität, Jena (Juni 1996)*
6. Rekombinante Antikörperfragmente in Diagnostik und Therapie.  
*Seminarreihe der Abteilung Hämatologie und Onkologie des Zentrums für Innere Medizin der Philipps-Universität, Marburg (1997)*
7. „Naïve“ Antikörperbibliotheken.  
*18. Sitzung des DECHEMA-Arbeitsausschusses „Grundlagen der Stoffproduktion“, Frankfurt/Main (September 1998)*
8. Bispezifische Antikörperfragmente für die Tumorthherapie.

*Seminarreihe des Instituts für Physiologische Chemie der Philipps-Universität, Marburg (Mai 1999)*

9. Bispecific single-chain diabodies: Developments for immunotherapy and gene therapy of cancer

*Seminarreihe des Instituts für Biologie I, Rheinisch-Westfälische Technische Hochschule Aachen (April 2000)*

10. Targeting of vectors and effectors with recombinant bispecific antibodies.

*Gene therapy seminar series am Gene Therapy Center at the University of Alabama at Birmingham (März 2001).*

11. Vector and effector targeting with bispecific recombinant antibodies.

*Cold Spring Harbor Laboratory Meeting on 'Vector Targeting Strategies for Therapeutic Gene Delivery' (März 2001).*

12. Rekombinante bispezifische Antikörper für die Behandlung von Krebs.

*Immunologisches Kolloquium, Institut für Immunologie, Universität Kiel (Juni 2001).*

13. Einsatz rekombinanter Antikörperfragmente in der Medizin.

*Journal Club, Aventis Behring, Marburg (September 2001).*

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No. 38005-0121

*In re* patent application of

Robert KONTERMANN et al.

Serial No. US 09/288,719

Group Art Unit: 1632

Filed: April 09, 1999

Examiner: A. Beckerleg

For: SINGLE-CHAIN MULTIPLE ANTIGEN-BINDING MOLECULE, ITS  
PREPARATION AND USE

**EXHIBIT C**

**Fusogenic peptides cited in**

**DE 196 49 645 A1**

The fusogenic peptides disclosed in DE 196 49 645.4 and listed on page 10, lines 30-64 and comprise for example:

- (1) peptide containing the peptide GLFEALLELLESLWELLLEA (Gottschalk *et al.*, Gene Ther. 3, 448 (1996));
- (2) peptide containing the peptide AALAEA[LAEA]<sub>4</sub>LAAAGC (Acm) (Wang *et al.*, Technol. Advances in Vector Syst. For Gene Ther., May 6-7, 1996, Coronado, IBC Conference);
- (3) peptide containing the peptide FAGV-VLAGAALGVAAAAQI of the fusion protein of measles-virus (Yeagle *et al.*, Biochem. Biophys. Acta 1065, 49 (1991));
- (4) peptide containing the peptide GLFGAIAGFIEGGWWGMIDG of the HA2 proteins of Influenza A (Lüneberg *et al.*, J. Biol. Chem. 270, 27606 (1995));
- (5) peptide containing the peptide GLFGAIAGFIENGWEGMIDG GLFGAIAGFIENGWEGMIDG (Burger *et al.*, Biochem. 30, 11173 (1991)) or the peptide GLFGAIAGFIE; ALFGAIAGFIE;

LFLGAIAGFIE; LLLGAIAGFIE; LILGAIAGFIE; GIFGAIAGFIE;  
GLLGAIAGFIE; GLFAAIAGFIE; GLFEAIAGFIE;  
GLFGAMAGFIE; GLFGAIAGLIE or the peptide GLFGAIAGFIV  
(Steinhauer *et al.*, J. Virol. 69, 6643 (1995));

- (6) the peptide GLFEAIAEFIEGGWEGLIEG; and
- (7) the peptide GLLEALAELEGGWEGLLEG (Ishiguro *et al.*, Biochem. 32, 9792 (1993)).

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

**Attorney Docket No. 38005-0121**

*In re* patent application of  
Robert KONTERMANN et al.

Serial No. US 09/288,719

Group Art Unit: 1632

Filed: April 09, 1999

Examiner: A. Beckerleg

For: SINGLE-CHAIN MULTIPLE ANTIGEN-BINDING MOLECULE, ITS  
PREPARATION AND USE

**EXHIBIT D**

**Target cell specific ligands cited in  
DE 196 49 645 A1**

The target cell specific ligands disclosed in DE 196 49 645.4 and listed on page 3, line 46 through page 9, line 63 comprise inter alia:

- (1) antibody fragments directed against membrane structures of endothelial cells such as, for example, Burrows *et al.* (Pharmac. Ther. 64, 155 (1994), Hughes *et al.* (Cancer Res. 49, 6214 (1989) and Murayama *et al.* (PNAS-USA 87, 5744 (1990)) specially antibodies against VEGF-receptors. (disclosed in DE 196 49 645 A1, p. 5, lines 19-22);
- (2) antibodies or antibody fragments directed against membrane structures of immune cells, such as described in Powelson *et al.*, Biotech. Adv. 11, 725 (1993) or antibodies or antibody fragments that bind with their antigen binding part the FC- $\gamma$ , FC- $\epsilon$  or FC- $\mu$  (Rojanasakul et al. Pharm. Res. 11, 1731 (1994), (disclosed in DE 196 49 645 A1, p. 5, lines 50-61);

- (3) antibodies or antibody fragments directed against membrane structures of muscle cells, such as the antibody 10F3, antibody against actin, antibody against angiotensin II receptors or antibodies against receptors of growth factors (disclosed in DE 196 49 645 A1, p. 6, lines 48-56);
- (4) antibodies or antibody fragments directed against membrane structures of tumor cells, such antibodies are described in Sedlacek *et al.*, Contrib. to Oncol. 32, Karger Publisher, Munich (1998) and Contrib. to Oncol. 43, Karger Publisher, Munich (1992) (disclosed in DE 196 49 645 A1, page 9, lines 50-54).

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No. 38005-0121

*In re* patent application of

Robert KONTERMANN et al.

Serial No. US 09/288,719

Group Art Unit: 1632

Filed: April 09, 1999

Examiner: A. Beckerleg

For: SINGLE-CHAIN MULTIPLE ANTIGEN-BINDING MOLECULE, ITS  
PREPARATION AND USE

**EXHIBIT E**

**Gene construct-specific ligands**

Such ligands comprise:

1. Anti-Fc- $\gamma$  receptor I (specific ligand for NK cells, macrophages, etc.) Tempest,  
P.R., Submitted to the National Center for Biological Resources, (NCBI, accessi-  
ble through <http://www.ncbi.nlm.nih.gov/>) 30-JUN-1995,

VH-Version: Accession number: Z50001.1 GI:895695

1 cagggtccaac tgcaggagag cgggtccaggt cttgtgagac ctagccagac  
cctgagcctg

61 acctgcaccg tgtctggctt cattttcagc gacaattaca tgtattgggt ga-  
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121 cctggacgag gtcttgagtg gattggaacc attagtgatg gtggtagtta  
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181 cctgacagtg tgaaggggag agtgacaatg ctgagagaca ccagcaagaa  
ccagttcagc

241 ctgagactca gcagcgtgac agccgccgac accgcggtct attattgtgc  
aagaggctac

301 tataggtacg agggggctat ggactactgg ggccaagggt ccttggtcac  
cgtctcctca

1/1	31/11
61/21	91/31

cag gtc caa ctg cag gag agc ggt cca ggt ctt gtg aga cct agc  
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gac aat tac atg tat tgg gtg aga cag cca

Q	V	Q	L	Q	E	S	G	P	G	L	V	R	P	S	Q
T	L	S	L	T	C	T	V	S	G	F	I	F	S	D	N
Y	M	Y	W	V	R	Q	P								

  

121/41	151/51
181/61	211/71

cct gga cga ggt ctt gag tgg att gga acc att agt gat ggt ggt  
agt tac acc tac tat cct gac agt gtg aag ggc aga gtg aca atg  
ctg aga gac acc agc aag aac cag ttc agc

P	G	R	G	L	E	W	I	G	T	I	S	D	G	G	S
Y	T	Y	Y	P	D	S	V	K	G	R	V	T	M	L	R
D	T	S	K	N	Q	F	S								

  

241/81	271/91
301/101	331/111

ctg aga ctc agc agc gtg aca gcc gcc gac acc gcg gtc tat tat  
tgt gca aga ggc tac tat agg tac gag ggg gct atg gac tac tgg  
ggc caa ggg tcc ttg gtc acc gtc tcc tca

L	R	L	S	S	V	T	A	A	D	T	A	V	Y	Y	C
A	R	G	Y	Y	R	Y	E	G	A	M	D	Y	W	G	Q
G	S	L	V	T	V	S	S								

VL-Version: Accession number: Z50002.1 GI:895696

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61 atcacctgta agtccagtca aagtgtttta tacagttcaa atcagaagaa  
ctacttgcc

121 tgggtaccagc agaagccagg taaggctcca aagctgctga tctactgggc  
atccactagg

181 gaatctggtg tgccaagcag attcagcggg agcggtagcg gtaccgactt  
caccttcacc

241 atcagcagcc tccagccaga ggacatcgcc acctactact gccatcaata  
cctctcctcg

301 tggagcttcg gccaaaggac caaggtggaa atcaaa

1/1	31/11
61/21	91/31



gac atc cag ctg acc cag agc cca agc agc ctg agc gcc agc gtg  
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tac agt tca aat cag aag aac tac ttg gcc  
  
D I Q L T Q S P S S L S A S V G  
D R V T I T C K S S Q S V L Y S  
S N Q K N Y L A  
  
121/41 151/51  
181/61 211/71  
  
tgg tac cag cag aag cca ggt aag gct cca aag ctg ctg atc tac  
tgg gca tcc act agg gaa tct ggt gtg cca agc aga ttc agc ggt  
agc ggt agc ggt acc gac ttc acc ttc acc  
  
W Y Q Q K P G K A P K L L I Y W  
A S T R E S G V P S R F S G S G  
S G T D F T F T  
  
241/81 271/91  
301/101 331/111  
  
atc agc agc ctc cag cca gag gac atc gcc acc tac tac tgc cat  
caa tac ctc tcc tcg tgg acg ttc ggc caa ggg acc aag gtg gaa  
atc aaa  
  
I S S L Q P E D I A T Y Y C H Q  
Y L S S W T F G Q G T K V E I K

2. anti-CD3 (specific ligand for cytotoxic T lymphocytes) Gilliland, L.K., et al.  
Tissue Antigens 47 (1), 1-20 (1996) Sequence database: National Center for  
Biological Resources, (NCBI, accessible through <http://www.ncbi.nlm.nih.gov/>)

VH-Version Accession number AF000359.1 GI:2522357

58 cag  
61 gtcaagctgc agcagtcggt ttctgaacta gggaaacctg gggcctcagt  
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gaagcct  
181 ggagaaagcc tgcagtggat aggaaatggt tatgggtggaa atgggtgtac aagcta-  
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301 gaactcagca gcctgacatc tgaggattct gccatctatt actgtgcaag  
aaggccggtg  
361 gcgacgggcc atgctatgga ctactggggt caggggatcc aagttaccgt ctctca

1/1	31/11
61/21	91/31

cag gtc aag ctg cag cag tcc ggt tct gaa cta ggg aaa cct ggg gcc  
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tat att tct tgg gtg aaa cag aag

Q	V	K	L	Q	Q	S	G	S	E	L	G	K	P	G	A	S
V	K	L	S	C	K	T	S	G	Y	I	F	T	D	H	Y	I
S	W	V	K	Q	K											

121/41	151/51
181/61	211/71

cct gga gaa agc ctg cag tgg ata gga aat gtt tat ggt gga aat ggt  
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P	G	E	S	L	Q	W	I	G	N	V	Y	G	G	N	G	G
T	S	Y	N	Q	K	F	Q	G	K	A	T	L	T	V	D	K
I	S	S	T	A	Y											

241/81	271/91
301/101	331/111

atg gaa ctc agc agc ctg aca tct gag gat tct gcc atc tat tac tgt  
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ggg atc caa gtt acc gtc tcc tca

M	E	L	S	S	L	T	S	E	D	S	A	I	Y	Y	C	A
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Q	V	T	V	S	S											

**VL-Version Accession number AF000358.1 GI:2522355**

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181 aaggaggctc caagggtctt catcaagtat gcttcgcagt ccattcctgg  
gatccctcc

241 agattcagtg gcagtgggtc ggaaacagat ttcactctca gcatcaataa  
cctggagcct

301 gatgatatcg gaatttatta ctgtcaacaa agtagaagct ggctgtcac  
gttcggtcct

361 ggcaccaagc tggagataaa a

1/1	31/11
61/21	91/31

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gaa aga gtc aca atg acc tgt aag acc agt cag aat att ggc aca atc  
tta cac tgg tat cac caa aaa cca  
D I V L T Q T P A T L S L I P G E  
R V T M T C K T S Q N I G T I L H  
W Y H Q K P  
121/41 151/51  
181/61 211/71  
aag gag gct cca agg gct ctc atc aag tat gct tcg cag tcc att cct  
ggg atc ccc tcc aga ttc agt ggc agt ggt tcg gaa aca gat ttc act  
ctc agc atc aat aac ctg gag cct  
K E A P R A L I K Y A S Q S I P G  
I P S R F S G S G S E T D F T L S  
I N N L E P  
241/81 271/91  
301/101  
gat gat atc gga att tat tac tgt caa caa agt aga agc tgg cct gtc  
acg ttc ggt cct ggc acc aag ctg gag ata aaa  
D D I G I Y Y C Q Q S R S W P V T  
F G P G T K L E I K

Antibody against ovarina, cerix, breast and colon carcinoma (ligands specific for membrane structures of tumor cells) Chen,P.F., et al., Hum. Antibodies Hybridomas 5 (3-4), 131-142 (1994), Sequence database: National Center for Biological Resources, (NCBI, accessible through <http://www.ncbi.nlm.nih.gov/>)

VH-Version Accession Number S77598.1 GI:998388

58 cag  
61 gtgcagctgg tgcagctctgg ggctgaggtg aagaagcctg gggcctcagt  
gaaggtttcc  
121 tgcaaggcat ctggatacac cttcaccagc tactatatgc actgggtcga  
acaggcccct  
181 ggacaagggc ttgagtggat gggaataatc aaccctagtg gtggtagcac  
aagctacgca  
241 cagaagttcc agggcagagt caccatgacc agggacacgt ccacgagcac agtcta-  
catg  
301 gagctgagca gcctgagatc tgaggacacg gccgtgtatt actgtgctag agag-  
gatggg  
361 cctacagcta tggccacaag ggccgaccta acgacaacat actactacta  
ctacggtatg  
421 gacgtctggg gccaaaggac c'acggtcacc gtctectca

1/1 31/11  
61/21 91/31

cag gtg cag ctg gtg cag tct ggg gct gag gtg aag aag cct ggg gcc  
tca gtg aag gtt tcc tgc aag gca tct gga tac acc ttc acc agc tac  
tat atg cac tgg gtc gaa cag gcc

Q V Q L V Q S G A E V K K P G A S  
V K V S C K A S G Y T F T S Y Y M  
H W V E Q A

121/41 151/51  
181/61 211/71

cct gga caa ggg ctt gag tgg atg gga ata atc aac cct agt ggt ggt  
agc aca agc tac gca cag aag ttc cag ggc aga gtc acc atg acc agg  
gac acg tcc acg agc aca gtc tac

P G Q G L E W M G I I N P S G G S  
T S Y A Q K F Q G R V T M T R D T  
S T S T V Y

241/81 271/91  
301/101 331/111

atg gag ctg agc agc ctg aga tct gag gac acg gcc gtg tat tac tgt  
gct aga gag gat ggg cct aca gct atg gcc aca agg gcc gac cta acg  
aca aca tac tac tac tac tac ggt

M E L S S L R S E D T A V Y Y C A  
R E D G P T A M A T R A D L T T T  
Y Y Y Y Y G

361/121 391/131

atg gac gtc tgg ggc caa ggg acc acg gtc acc gtc tcc tca

M D V W G Q G T T V T V S S

# VL-VERSION Accession Number S77599.1 GI: 998390

61 gagctgacac agccaccctc ggtgtcagtg tccctaggac agatggccag gat-  
cacctgc

121 tctggagaag cattgccaaa aaaatatgct tattggtacc agcagaagcc  
aggccagttc

181 cctgtgctgg tgatatataa agacagcgag agggcctcag ggatccctga  
gcgattctct

241 ggctccagct cagggacaat agtcacattg accatcagtg gagtccaggc agaa-  
gacgag

301 gctgactatt actgtctatc agcagacagc agtggtactt atggcgggtg  
gttcggcgga

361 gggaccaagc tgaccgtcct a

1/1

31/11

61/21

91/31

gag ctg aca cag cca ccc tcg gtg tca gtg tcc cta gga cag atg gcc  
agg atc acc tgc tct gga gaa gca ttg cca aaa aaa tat gct tat tgg  
tac cag cag aag cca ggc cag ttc

E L T Q P P S V S V S L G Q M A R  
I T C S G E A L P K K Y A Y W Y Q  
Q K P G Q F

121/41

151/51

181/61

211/71

cct gtg ctg gtg ata tat aaa gac agc gag agg gcc tca ggg atc cct  
gag cga ttc tct ggc tcc agc tca ggg aca ata gtc aca ttg acc atc  
agt gga gtc cag gca gaa gac gag

P V L V I Y K D S E R A S G I P E  
R F S G S S S G T I V T L T I S G  
V Q A E D E

241/81

271/91

301/101

gct gac tat tac tgt cta tca gca gac agc agt ggt act tat ggc ggg  
tgg ttc ggc gga ggg acc aag ctg acc gtc cta

A D Y Y C L S A D S S G T Y G G W  
F G G G T K L T V L

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

**Attorney Docket No. 38005-0121**

*In re* patent application of

Robert KONTERMANN et al.

Serial No. US 09/288,719

Group Art Unit: 1632

Filed: April 09, 1999

Examiner: A. Beckerleg

For: SINGLE-CHAIN MULTIPLE ANTIGEN-BINDING MOLECULE, ITS  
PREPARATION AND USE

**EXHIBIT F**

**Gene construct-specific ligands cited in  
DE 196 49 645 A1**

The gene construct-specific ligands disclosed in DE 196 49 645.4 and listed on page 11, line 55 through page 13, line 40 comprise inter alia:

(1) antibodies directed against epitopes newly introduced into DNA such as antibodies directed against methylated DNA, antibodies against O<sup>6</sup>-ethyl deoxyguanosin, antibodies against N<sup>5</sup>-methyl-N5-formyl-2,5,6,-triamino-4-hydroxypyrimidine, antibodies against N7-ethyl guanine, antibodies against O<sup>6</sup>-methyl-2'-deoxyguanosine, antibodies against O<sup>6</sup>-ethyl-2'-deoxyguanosine, antibodies against O<sup>6</sup>-N-butyl-2'- deoxyguanosine, antibodies against O<sup>6</sup>-isopropyl-2'-deoxyguanosine, antibodies against O<sup>4</sup>-methyl-2'-deoxyguanosine or antibodies against O<sup>4</sup>-ethyl-2'-deoxyguanosine, antibodies against methylated DNA, especially against N<sup>6</sup>-methylated adenin.

(2) antibodies directed against envelope proteins or viruses such as for example

- murine leukemia virus, the antibody being preferably directed against envelope proteins gp70 and p15,
- HIV,
- herpes simplex virus, the antibody being preferably directed against glycoprotein B, glycoprotein H, glycoprotein L,
- cytomegalovirus, the antibody being preferably directed against glycoprotein B (gpB),
- adanoassociated virus,
- minute virus of mice,
- antibodies against adanoassociated virus, the antibody being preferably directed against Cap- and Rep-proteins,
- cytomegalovirus, the antibody being preferably directed against glycoprotein B (gpB),

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

**Attorney Docket No. 38005-0121**

*In re* patent application of

Robert KONTERMANN et al.

Serial No. US 09/288,719

Group Art Unit: 1632

Filed: April 09, 1999

Examiner: A. Beckerleg

For: SINGLE-CHAIN MULTIPLE ANTIGEN-BINDING MOLECULE, ITS  
PREPARATION AND USE

**EXHIBIT G**

**Gene construct-specific ligands**

Such antibodies against DNA comprise inter alia:

Anti-DNA antibodies (ligands specific for DNA) Jang, Y.J. et al., Mol. Immunol. 33 (2), 197-210 (1996) Sequence database: National Center for Biological Resources, (NCBI, accessible through <http://www.ncbi.nlm.nih.gov/>) Accession number 96215469

VH-Version Accession number U23046.1 GI:736417

```
1  caggtccaac tacagcagcc tgggtgctgag cttgtgaagc ctggggcctc agt-  
gaagctg  
61  tcctgcaagg cttctggcta cactttcacc aggttctgga taaactgggt gaggca-  
gagg  
121 cctggacaag gccttgagtg gattggaaat atttatcctg gtagtagtag tat-  
taactac  
181 aatgagaagt tcaagaacaa ggccacactg actgtagaca catcctccag ca-  
cagcctac  
241 atgcagctca gcagcctgac atctgacgac tctgcggtct attattgtgc aa-  
gacggcgg  
301 tataggctct cctatgctat ggactactgg ggtcaaggaa cctcagtcac  
cgtctcctca
```



1/1	31/11
61/21	91/31

cag gtc caa cta cag cag cct ggt gct gag ctt gtg aag cct ggg gcc  
tca gtg aag ctg tcc tgc aag gct tct ggc tac act ttc acc agg ttc  
tgg ata aac tgg gtg agg cag agg

Q	V	Q	L	Q	Q	P	G	A	E	L	V	K	P	G	A	S
V	K	L	S	C	K	A	S	G	Y	T	F	T	R	F	W	I
N	W	V	R	Q	R											

  

121/41	151/51
181/61	211/71

cct gga caa ggc ctt gag tgg att gga aat att tat cct ggt agt agt  
agt att aac tac aat gag aag ttc aag aac aag gcc aca ctg act gta  
gac aca tcc tcc agc aca gcc tac

P	G	Q	G	L	E	W	I	G	N	I	Y	P	G	S	S	S
I	N	Y	N	E	K	F	K	N	K	A	T	L	T	V	D	T
S	S	S	T	A	Y											

  

241/81	271/91
301/101	331/111

atg cag ctc agc agc ctg aca tct gac gac tct gcg gtc tat tat tgt  
gca aga cgg cgg tat agg tcc tcc tat gct atg gac tac tgg ggt caa  
gga acc tca gtc acc gtc tcc tca

M	Q	L	S	S	L	T	S	D	D	S	A	V	Y	Y	C	A
R	R	R	Y	R	S	S	Y	A	M	D	Y	W	G	Q	G	T
S	V	T	V	S	S											

VL-Version Accession number U23047.1 GI:736419

1 gaaacaactg tgacccagtc tccagcatcc ctgtccgtgg ctacaggaga aaaagt-  
cact

61 atcagatgca taaccaacac tgatattgat gatgatatga actggtacca gcagaag-  
cca

121 ggggaacctc ctaagctcct tatttcagaa ggcaatactc ttcgtcctgg  
agtcccatcc

181 cgattctcca gcagtggcta tggcactgat tttgttttta caattgaaaa  
cacgctctca

241 gaagatgttg cagattactg ctgtttgcaa agtgataaca tgcctctcac  
gttcggtgct

301 gggaccaagc tggagctgaa a

1/1	31/11
61/21	91/31

gaa aca act gtg acc cag tct cca gca tcc ctg tcc gtg gct aca gga  
gaa aaa gtc act atc aga tgc ata acc aac act gat att gat gat gat  
atg aac tgg tac cag cag aag cca

E T T V T Q S P A S L S V A T G E  
K V T I R C I T N T D I D D D M N  
W Y Q Q K P

121/41

151/51

181/61

211/71

ggg gaa cct cct aag ctc ctt att tca gaa ggc aat act ctt cgt cct  
gga gtc cca tcc cga ttc tcc agc agt ggc tat ggc act gat ttt gtt  
ttt aca att gaa aac acg ctc tca

G E P P K L L I S E G N T L R P G  
V P S R F S S S G Y G T D F V F T  
I E N T L S

241/81

271/91

301/101

gaa gat gtt gca gat tac tgc tgt ttg caa agt gat aac atg cct ctc  
acg ttc ggt gct ggg acc aag ctg gag ctg aaa

E D V A D Y C C L Q S D N M P L T  
F G A G T K L E L K

# 1. anti-herpes glycoprotein B (ligands specific for herpes viral coat proteins)

Ohlin,M. et al., Mol. Immunol. 33 (1), 47-56 (1996), sequence dadabase: National Center for Biological Resources, (NCBI, accessible through <http://www.ncbi.nlm.nih.gov/>)

VH-Version Accession number L37310.1 GI:845513

1 cagctgcagc tgggtggagtc tgggggaggc gtggtccagc ctgggaggtc cttga-  
gactc

61 tctctgtgcag cctctggggtt cattttcagt gagtatgata tgcactgggt  
cgcaggct

121 ccaggcaagg ggctgcagtg ggtggcagtt atatcagttg atggaagtga taaa-  
cactac

181 gcagactccg tgaagggccg attcaccatc tccagagaca attcccagaa  
catgttggtt

241 ctacaaatgg acagcctgag aggtgacgac acggctgttt attattgtgc gaga-  
gatgga

301 aaaagtttga atggttattc cggcttgatt gactactggg gccagggatc cttagt-  
cacc

361 gtctcctca

1/1 31/11  
61/21 91/31

cag ctg cag ctg gtg gag tct ggg gga ggc gtg gtc cag cct ggg agg  
tcc ttg aga ctc tcc tgt gca gcc tct ggg ttc att ttc agt gag tat  
gat atg cac tgg gtc cgc cag gct

Q L Q L V E S G G G V V Q P G R S  
L R L S C A A S G F I F S E Y D M  
H W V R Q A

121/41 151/51  
181/61 211/71

cca ggc aag ggg ctg cag tgg gtg gca gtt ata tca gtt gat gga agt  
gat aaa cac tac gca gac tcc gtg aag ggc cga ttc acc atc tcc aga  
gac aat tcc cag aac atg ttg ttt

P G K G L Q W V A V I S V D G S D  
K H Y A D S V K G R F T I S R D N  
S Q N M L F

241/81 271/91  
301/101 331/111

cta caa atg gac agc ctg aga ggt gac gac acg gct gtt tat tat tgt  
gcg aga gat gga aaa agt ttg aat ggt tat tcc ggc ttg att gac tac  
tgg ggc cag gga tcc tta gtc acc

L Q M D S L R G D D T A V Y Y C A  
R D G K S L N G Y S G L I D Y W G  
Q G S L V T

361/121

gtc tcc tca  
V S S

# VL-Version Accession number L37301.1 GI:845517

1 gaaattgtgt tgacgcagtc tccagccacc ctgtctttgt ctccagggga aa-  
gagccacc

61 ctctcctgca gggccagtca gagtgttggc agctccttag cctggtacca aca-  
gaaacct

121 ggccaggtct ccaggctcct cgtctatgat acatccaaca gggccactgg  
catcccagcc

181 aggttcaatg gcagtgggtc tgggacagac ttcactctca ccatcagcag ccta-  
gagcct

241 gaagattttg cagattatta ctgtcaacag cgaagcgagt ggcctctcac  
tttcggcgga

301 gggaccaagg tggagatcaa a

1/1 31/11  
61/21 91/31

gaa att gtg ttg acg cag tct cca gcc acc ctg tct ttg tct cca ggg  
gaa aga gcc acc ctc tcc tgc agg gcc agt cag agt gtt ggc agc tcc  
tta gcc tgg tac caa cag aaa cct

E I V L T Q S P A T L S L S P G E  
R A T L S C R A S Q S V G S S L A  
W Y Q Q K P

121/41 151/51  
181/61 211/71

ggc cag gct ccc agg ctc ctc gtc tat gat aca tcc aac agg gcc act  
ggc atc cca gcc agg ttc aat ggc agt ggg tct ggg aca gac ttc act  
ctc acc atc agc agc cta gag cct

G Q A P R L L V Y D T S N R A T G  
I P A R F N G S G S G T D F T L T  
I S S L E P

241/81 271/91  
301/101

gaa gat ttt gca gat tat tac tgt caa cag cga agc gag tgg cct ctc  
act ttc ggc gga ggg acc aag gtg gag atc aaa

E D F A D Y Y C Q Q R S E W P L T  
F G G G T K V E I K

2. Anti-glycoprotein D (ligands specific for viral coat proteins of HSV-1) Schellens, G.A., Submitted to the sequence database of the National Center for Biological Resources, (NCBI, accessible through <http://www.ncbi.nlm.nih.gov/>) at 01-NOV-1993)

VH-Version Accession number X75537.1 GI:414141

1 gagtcgggac ctggcctggt gaaaccttct caatctctgt ccctcacctg  
cactgtctct

61 ggctcctcaa tcaccagtga ttatgcctgg acctggatct ggcagtttcc aggaaa-  
caaa

121 ctggagtgga tgggctacat aagctacatt ggtgccacta gctacaaccc  
ctctctccaa

181 agtcgaatct ctatcactcg agacacctcc aagaaccatt tcttctaca gttga-  
attct

241 gtgaccactg aggacacagc cacatattac tgtgcacgag aggggtcttg  
gttcttcggt

301 gtctggggcg cagggaccac ggtcaccgtc tcctca

1/1

31/11

61/21

91/31

gag tcg gga cct ggc ctg gtg aaa cct tct caa tct ctg tcc ctc acc  
tgc act gtc tct ggc tcc tca atc acc agt gat tat gcc tgg acc tgg  
atc tgg cag ttt cca gga aac aaa

E	S	G	P	G	L	V	K	P	S	Q	S	L	S	L	T	C
T	V	S	G	S	S	I	T	S	D	Y	A	W	T	W	I	W
Q	F	P	G	N	K											

121/41

151/51

181/61

211/71

ctg gag tgg atg ggc tac ata agc tac att ggt gcc act agc tac aac  
ccc tct ctc caa agt cga atc tct atc act cga gac acc tcc aag aac  
cat ttc ttc cta cag ttg aat tct

L	E	W	M	G	Y	I	S	Y	I	G	A	T	S	Y	N	P
S	L	Q	S	R	I	S	I	T	R	D	T	S	K	N	H	F
F	L	Q	L	N	S											

241/81

271/91

301/101

331/111

gtg acc act gag gac aca gcc aca tat tac tgt gca cga gag ggg tct  
tgg ttc ttc ggt gtc tgg ggc gca ggg acc acg gtc acc gtc tcc tca

V	T	T	E	D	T	A	T	Y	Y	C	A	R	E	G	S	W
F	F	G	V	W	G	A	G	T	T	V	T	V	S	S		

## VL-Version Accession number X75536.1 GI:414143

1 gtgatgaccc agtctccact ctccctgcct gtcagtcttg gagatcaagc  
ctccatctct

61 tgcagatcta gtcagagcct tgtacacact aatggaaaca cctattttaca  
ttggtacctg

121 cagaagccag gccagtctcc aaaggtcctg atctacaaag tttccaccgg  
attttctggg

181 gtcccagaca ggttcagtgg cagtggatca gggacagatt tcacattcaa gatcag-  
caga

241 gtggaggctg aggatctggg agtttatttc tgctctcaaa gtacatatgt  
tccatttcag

301 ttcgggtcgg ggacaaagtt ggaaataaaa cgg

1/1 31/11  
61/21 91/31

gtg atg acc cag tct cca ctc tcc ctg cct gtc agt ctt gga gat caa  
gcc tcc atc tct tgc aga tct agt cag agc ctt gta cac act aat gga  
aac acc tat tta cat tgg tac ctg

V M T Q S P L S L P V S L G D Q A  
S I S C R S S Q S L V H T N G N T  
Y L H W Y L

121/41 151/51  
181/61 211/71

cag aag cca ggc cag tct cca aag gtc ctg atc tac aaa gtt tcc acc  
cga ttt tct ggg gtc cca gac agg ttc agt ggc agt gga tca ggg aca  
gat ttc aca ttc aag atc agc aga

Q K P G Q S P K V L I Y K V S T R  
F S G V P D R F S G S G S G T D F  
T F K I S R

241/81 271/91  
301/101 331/111

gtg gag gct gag gat ctg gga gtt tat ttc tgc tct caa agt aca tat  
gtt cca ttc acg ttc ggc tcg ggg aca aag ttg gaa ata aaa cgg

V E A E D L G V Y F C S Q S T Y V  
P F T F G S G T K L E I K R

3. anti-hepatitis B virus S antigen (ligands specific for viral coat proteins of hepatitis B virus) Ryu,C.J., et al. , Gene 144 (2), 313-314 (1994) Sequence database: National Center for Biological Resources, (NCBI, accessible through <http://www.ncbi.nlm.nih.gov/>)

VH-Version Accession number L25332.1 GI:437012

1 gaggttcagc tgcaacaatc tggacctgag ctggtgaaac ctggggcctc agtgaa-  
gata

61 tcttgcaagg cttctggata tacattcact gactacaaca ttcagtgggt gaagca-  
gagc

121 catggaaaga gccttgagtg gattggatat atttatectt aactgggtgg  
tactggctac

181 agccagaagt tcaagagcaa ggccacattg actgtagaca atttctccag ca-  
cagcctat

241 atggaactcc gcagcctgac atctgaggac tctgcagtct attactgtgc aa-  
gaaactat

301 ggttacgacg agtctgctta ctggggccaa gggactctgg tcaactgtctc tgca

1/1	31/11
61/21	91/31
gag gtt cag ctg caa caa tct gga cct gag ctg gtg aaa cct ggg gcc tca gtg aag ata tcc tgc aag gct tct gga tat aca ttc act gac tac aac att cag tgg gtg aag cag agc	
E V Q L Q Q S G P E L V K P G A S V K I S C K A S G Y T F T D Y N I Q W V K Q S	
121/41	151/51
181/61	211/71
cat gga aag agc ctt gag tgg att gga tat att tat cct tac act ggt ggt act ggc tac agc cag aag ttc aag agc aag gcc aca ttg act gta gac aat ttc tcc agc aca gcc tat	
H G K S L E W I G Y I Y P Y T G G T G Y S Q K F K S K A T L T V D N F S S T A Y	
241/81	271/91
301/101	331/111
atg gaa ctc cgc agc ctg aca tct gag gac tct gca gtc tat tac tgt gca aga aac tat ggt tac gac gag tct gct tac tgg ggc caa ggg act ctg gtc act gtc tct gca	
M E L R S L T S E D S A V Y Y C A R N Y G Y D E S A Y W G Q G T L V T V S A	

**VL-Version Accession number L25333.1 GI:437013**

1 gacattgtgc tgacccaatc tccagcttct ttggctgtgt ctctagggca  
gagggccacc

61 atctcctgca gagccagcga aagtgttgat aattatggca ttagttttat  
gaactggttc

121 caacagaaac caggacagcc acccaaactc ctcatctata ctgcatccaa ccaag-  
gatcc

181 ggggtccctg ccaggtttag tggcagtggg tctgggacag acttcagcct caa-  
catccat

241 cctatggagg tggatgatac tgcaatgtat ttctgtcagc aaactaagga  
ggttccgtac

301 acgttcggag gggggaccaa gctggaaata aaacgg

1/1 31/11  
61/21 91/31

gac att gtg ctg acc caa tct cca gct tct ttg gct gtg tct cta ggg  
cag agg gcc acc atc tcc tgc aga gcc agc gaa agt gtt gat aat tat  
ggc att agt ttt atg aac tgg ttc

D I V L T Q S P A S L A V S L G Q  
R A T I S C R A S E S V D N Y G I  
S F M N W F

121/41 151/51  
181/61 211/71

caa cag aaa cca gga cag cca ccc aaa ctc ctc atc tat act gca tcc  
aac caa gga tcc ggg gtc cct gcc agg ttt agt ggc agt ggg tct ggg  
aca gac ttc agc ctc aac atc cat

Q Q K P G Q P P K L L I Y T A S N  
Q G S G V P A R F S G S G S G T D  
F S L N I H

241/81 271/91  
301/101 331/111

cct atg gag gtg gat gat act gca atg tat ttc tgt cag caa act aag  
gag gtt ccg tac acg ttc gga ggg ggg acc aag ctg gaa ata aaa cgg

P M E V D D T A M Y F C Q Q T K E  
V P Y T F G G G T K L E I K R

4. anti- anti-hepatitis C virus envelope protein (ligands specific for viral coat proteins of hepatitis C virus) Chan,S.W., J. Gen. Virol. 77 (Pt 10), 2531-2539 (1996), Sequence database: National Center for Biological Resources, (NCBI, accessible through <http://www.ncbi.nlm.nih.gov/>) Medline Accession numberr 97042287

VH-Version Accession number X97554.1 GI:1657322

2 atggcccag gtacagctgc agcagtcagg gggaggcctg gtcaagcctg ggggatccct  
61 gagactctcc tgtgcaggct ctggattcag catcgagac tatagcatga  
actgggtccg

121 ccaggctcca ggaaggggc tggagtgggt cgcgtccatc agtcctgata  
gtgtttatag

181 acactatgca gactcactga ggggccgatt caccatttcc agagacaacg ccag-  
gaactc

241 actgtttctg caattgacca ggctgacagc cgacgacacg gctgtgtatt  
ggtgtgcgag

301 acaccgacct acatatggtg tctactatta tggtatggac gtctggggcc  
acgggaccac



361 ggtcaccgtg tcga

1/1

31/11

61/21

91/31

atg gcc cag gta cag ctg cag cag tca ggg gga ggc ctg gtc aag cct  
ggg gga tcc ctg aga ctc tcc tgt gca ggc tct gga ttc agc atc gga  
gac tat agc atg aac tgg gtc cgc

M	A	Q	V	Q	L	Q	Q	S	G	G	G	L	V	K	P	G
G	S	L	R	L	S	C	A	G	S	G	F	S	I	G	D	Y
S	M	N	W	V	R											

121/41

151/51

181/61

211/71

cag gct cca ggg aag ggg ctg gag tgg gtc gcg tcc atc agt cct gat  
agt gtt tat aga cac tat gca gac tca ctg agg ggc cga ttc acc att  
tcc aga gac aac gcc agg aac tca

Q	A	P	G	K	G	L	E	W	V	A	S	I	S	P	D	S
V	Y	R	H	Y	A	D	S	L	R	G	R	F	T	I	S	R
D	N	A	R	N	S											

241/81

271/91

301/101

331/111

ctg ttt ctg caa ttg acc agg ctg aca gcc gac gac acg gct gtg tat  
tgg tgt gcg aga cac cga cct aca tat ggt gtc tac tat tat ggt atg  
gac gtc tgg ggc cac ggg acc acg

L	F	L	Q	L	T	R	L	T	A	D	D	T	A	V	Y	W
C	A	R	H	R	P	T	Y	G	V	Y	Y	Y	G	M	D	V
W	G	H	G	T	T											

361/121

gtc acc gtg tcg a

V T V S

VL-Version Accession number X97555.1 GI:1657328

1 gacatccaga tgacccagtc tccatcctcc ctgtctgcat ctgtagggga cagagt-  
cacc

61 atcacttgtc gggcgagtca gggtattagc agctggtttag cctgggtatca gcagaag-  
cca

121 gggaaagccc ctaagctcct gatctatgct gcatccagtt tgcaaagtgg  
ggtcccatca

181 aggttcagcg gcagtggatc tgggacacat ttcactctca ctatcagcag  
cctgcagcct

241 gaagactttg caacttacta ttgtcaacag gctaacagtt tccccctcac  
tttcggcgga

301 gggaccaagg tggaaatcaa acgtgcggcc

1/1

31/11

61/21

91/31

gac atc cag atg acc cag tct cca tcc tcc ctg tct gca tct gta ggg  
gac aga gtc acc atc act tgt cgg gcg agt cag ggt att agc agc tgg  
tta gcc tgg tat cag cag aag cca

D	I	Q	M	T	Q	S	P	S	S	L	S	A	S	V	G	D
R	V	T	I	T	C	R	A	S	Q	G	I	S	S	W	L	A
W	Y	Q	Q	K	P											

121/41

151/51

181/61

211/71

ggg aaa gcc cct aag ctc ctg atc tat gct gca tcc agt ttg caa agt  
ggg gtc cca tca agg ttc agc ggc agt gga tct ggg aca cat ttc act  
ctc act atc agc agc ctg cag cct

G	K	A	P	K	L	L	I	Y	A	A	S	S	L	Q	S	G
V	P	S	R	F	S	G	S	G	S	G	T	H	F	T	L	T
I	S	S	L	Q	P											

241/81

271/91

301/101

gaa gac ttt gca act tac tat tgt caa cag gct aac agt ttc ccc ctc  
act ttc ggc gga ggg acc aag gtg gaa atc aaa cgt gcg gcc

E	D	F	A	T	Y	Y	C	Q	Q	A	N	S	F	P	L	T
F	G	G	G	T	K	V	E	I	K	R	A	A				

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

**Attorney Docket No. 38005-0121**

*In re* patent application of

Robert KONTERMANN et al.

Serial No. US 09/288,719

Group Art Unit: 1632

Filed: April 09, 1999

Examiner: A. Beckerleg

For: SINGLE-CHAIN MULTIPLE ANTIGEN-BINDING MOLECULE, ITS  
PREPARATION AND USE

**EXHIBIT H**

**Sequences of single chain multiple antigen binding molecules**

Examples of single chain multiple antigen binding molecules (ScMAB) comprise inter alia:

1. ScMAB anti-Tumor x anti CD3 (based on sequences with the accession number S77598.1 / S77599.1 and AF000359.1 / AF000358.1) for the retargeting of cytotoxic T lymphocytes to tumor cells (anti-CD3 antibody specific for cytotoxic T lymphocytes, Gilliland, L.K., et al. Tissue Antigens 47 (1), 1-20 (1996) Sequence database: National Center for Biological Resources; anti-tumor antibody (against ovarina, cerix, breast and colon carcinoma, i.e. specific for membrane structures of tumor cells, Chen, P.F., et al., Hum. Antibodies Hybridomas 5 (3-4), 131-142 (1994), sequence database: National Center for Biological Resources, (NCBI, accessible through <http://www.ncbi.nlm.nih.gov/>)).

Structure of the ScMAB:

#	Nucleic	Function
	Acid	
1-66		pelB signal sequence
67-468		VH anti-Tumor
469-483		Linker L1

484-804	VL anti-CD3
805-849	Peptide Linker P
850-1209	VH anti-CD3
1210-1224	Linker L2
1225-1545	VL anti-Tumor
1546-1548	Stop codon

ATGAAATACCTATTGCCTACGGCAGCCGCTGGATTGTTATTACTCGCGGC  
 CCAGCCGGCCATGGCCcaggtgcagctggtgcagctctggggctgaggtga  
 agaagcctggggcctcagtgaaaggtttcctgcaaggcatctggatacacc  
 ttcaccagctactatatgcactgggtcgaacaggcccctggacaagggct  
 tgagtggatgggaataatcaaccctagtggtagcacaagctacgcac  
 agaagttccagggcagagtcaccatgaccaggacacgtccacgagcaca  
 gtctacatggagctgagcagcctgagatctgaggacacggcgtgtatta  
 ctgtgctagagaggatgggcctacagctatggccacaagggccgacctaa  
 cgacaacatactactactactacggtatggacgtctggggccaagggacc  
 acggtcaccgtctcctcaGGCGGTGGCGGATCGgacatagtgtgactca  
 gactccagccactctgtctctaattcctggagaaagagtcacaatgacct  
 gtaagaccagtcagaatattggcacaatcttacactggtatcaccaaaaa  
 ccaaaggaggctccaagggctctcatcaagtatgcttcgcagtcattcc  
 tgggatcccctccagattcagtggtggttcggaaacagatttcactc  
 tcagcatcaataacctggagcctgatgatatcggaatttattactgtcaa  
 caaagtagaagctggcctgtcacgttcggtcctggcaccaagctggagat  
 aaaaGGAGGCGGTGGCAGCGGTGGGCGCGCCTCGGGCGGAGGTGGCTCAc  
 aggtcaagctgcagcagtcggttctgaactagggaacctggggcctca  
 gtgaaactgtcctgcaagacttcaggctacatattcacagatcactatat  
 ttcttgggtgaaacagaagcctggagaaagcctgcagtggtataggaaatg  
 tttatgggtggaaatgggtgtacaagctacaatcaaaaattccagggcaag  
 gccacactgactgtagataaaatctctagcacagcctacatggaactcag  
 cagcctgacatctgaggattctgccatctattactgtgcaagaaggccgg  
 tagcgacgggcatgctatggactactggggtcaggggatccaagttacc  
 gtctcctcaGGAGGCGGGGTTCGgatatcgagctcccaccctcggtgtc  
 agtgtccctaggacagatggccaggatcacctgctctggagaagcattgc  
 caaaaaaatatgcttattggtaccagcagaagccaggccagttccctgtg

ctggtgatataataagacagcgagagggcctcagggatccctgagcgatt  
ctctggctccagctcagggacaatagtcacattgaccatcagtggagtcc  
aggcagaagacgagggctgactattactgtctatcagcagacagcagtggt  
acttatggcgggtgggttcggcggagggacccaagctgaccgtcctaTAA

1/1  
61/21

31/11  
91/31

ATG AAA TAC CTA TTG CCT ACG GCA GCC GCT GGA TTG TTA TTA CTC GCG  
GCC CAG CCG GCC ATG GCC cag gtg cag ctg gtg cag tct ggg gct gag  
gtg aag aag cct ggg gcc tca gtg

M K Y L L P T A A A G L L L L A A  
Q P A M A Q V Q L V Q S G A E V K  
K P G A S V

121/41  
181/61

151/51  
211/71

aag gtt tcc tgc aag gca tct gga tac acc ttc acc agc tac tat atg  
cac tgg gtc gaa cag gcc cct gga caa ggg ctt gag tgg atg gga ata  
atc aac cct agt ggt ggt agc aca

K V S C K A S G Y T F T S Y Y M H  
W V E Q A P G Q G L E W M G I I N  
P S G G S T

241/81  
301/101

271/91  
331/111

agc tac gca cag aag ttc cag ggc aga gtc acc atg acc agg gac acg  
tcc acg agc aca gtc tac atg gag ctg agc agc ctg aga tct gag gac  
acg gcc gtg tat tac tgt gct aga

S Y A Q K F Q G R V T M T R D T S  
T S T V Y M E L S S L R S E D T A  
V Y Y C A R

361/121  
421/141

391/131  
451/151

gag gat ggg cct aca gct atg gcc aca agg gcc gac cta acg aca aca  
tac tac tac tac tac ggt atg gac gtc tgg ggc caa ggg acc acg gtc  
acc gtc tcc tca GGC GGT GGC GGA

E D G P T A M A T R A D L T T T Y  
Y Y Y Y G M D V W G Q G T T V T V  
S S G G G G

481/161  
541/181

511/171  
571/191

TCG gac ata gtg ctg act cag act cca gcc act ctg tct cta att cct  
gga gaa aga gtc aca atg acc tgt aag acc agt cag aat att ggc aca  
atc tta cac tgg tat cac caa aaa

S D I V L T Q T P A T L S L I P G  
E R V T M T C K T S Q N I G T I L  
H W Y H Q K

601/201 631/211  
661/221 691/231

cca aag gag gct cca agg gct ctc atc aag tat gct tcg cag tcc att  
cct ggg atc ccc tcc aga ttc agt ggc agt ggt tcg gaa aca gat ttc  
act ctc agc atc aat aac ctg gag

P K E A P R A L I K Y A S Q S I P  
G I P S R F S G S G S E T D F T L  
S I N N L E

721/241 751/251  
781/261 811/271

cct gat gat atc gga att tat tac tgt caa caa agt aga agc tgg cct  
gtc acg ttc ggt cct ggc acc aag ctg gag ata aaa GGA GGC GGT GGC  
AGC GGT GGG CGC GCC TCG GGC GGA

P D D I G I Y Y C Q Q S R S W P V  
T F G P G T K L E I K G G G G S G  
G R A S G G

841/281 871/291  
901/301 931/311

GGT GGC TCA cag gtc aag ctg cag cag tcc ggt tct gaa cta ggg aaa  
cct ggg gcc tca gtg aaa ctg tcc tgc aag act tca ggc tac ata ttc  
aca gat cac tat att tct tgg gtg

G G S Q V K L Q Q S G S E L G K P  
G A S V K L S C K T S G Y I F T D  
H Y I S W V

961/321 991/331  
1021/341 1051/351

aaa cag aag cct gga gaa agc ctg cag tgg ata gga aat gtt tat ggt  
gga aat ggt ggt aca agc tac aat caa aaa ttc cag ggc aag gcc aca  
ctg act gta gat aaa atc tct agc

K Q K P G E S L Q W I G N V Y G G  
N G G T S Y N Q K F Q G K A T L T  
V D K I S S

1081/361 1111/371  
1141/381 1171/391

aca gcc tac atg gaa ctc agc agc ctg aca tct gag gat tct gcc atc  
tat tac tgt gca aga agg ccg gta gcg acg ggc cat gct atg gac tac  
tgg ggt cag ggg atc caa gtt acc

```

T   A   Y   M   E   L   S   S   L   T   S   E   D   S   A   I   Y
Y   C   A   R   R   P   V   A   T   G   H   A   M   D   Y   W   G
Q   G   I   Q   V   T

1201/401                               1231/411
1261/421                               1291/431

gtc tcc tca GGA GGC GGG GGT TCG gat atc gag ctg cca ccc tcg gtg
tca gtg tcc cta gga cag atg gcc agg atc acc tgc tct gga gaa gca
ttg cca aaa aaa tat gct tat tgg

V   S   S   G   G   G   G   S   D   I   E   L   P   P   S   V   S
V   S   L   G   Q   M   A   R   I   T   C   S   G   E   A   L   P
K   K   Y   A   Y   W

1321/441                               1351/451
1381/461                               1411/471

tac cag cag aag cca ggc cag ttc cct gtg ctg gtg ata tat aaa gac
agc gag agg gcc tca ggg atc cct gag cga ttc tct ggc tcc agc tca
ggg aca ata gtc aca ttg acc atc

Y   Q   Q   K   P   G   Q   F   P   V   L   V   I   Y   K   D   S
E   R   A   S   G   I   P   E   R   F   S   G   S   S   S   G   T
I   V   T   L   T   I

1441/481                               1471/491
1501/501                               1531/511

agt gga gtc cag gca gaa gac gag gct gac tat tac tgt cta tca gca
gac agc agt ggt act tat ggc ggg tgg ttc ggc gga ggg acc aag ctg
acc gtc cta TAA

S   G   V   Q   A   E   D   E   A   D   Y   Y   C   L   S   A   D
S   S   G   T   Y   G   G   W   F   G   G   G   T   K   L   T   V
L   *

```

2. ScMAB anti-Tumor x anti Anti-FC- $\gamma$  receptor I (based on sequences with the accession number Seq. S77598.1 / S77599.1 and Z50001.1 / Z50002.1) for the retargeting of NK cells and macrophages to tumor cells (Anti-FC- $\gamma$  receptor I-antibody specific for NK cells, macrophages, Tempest, P.R., submitted to the National Center for Biological Resources 30-JUN-1995; and anti-tumor antibody specific for ovarina, cerix, breast and colon carcinoma, i.e. specific for membrane structures of tumor cells, Chen,P.F., et al., Hum. Antibodies Hybridomas 5 (3-4), 131-142 (1994), Sequence dadabase: National Center for Biological Resources, (NCBI, accessible through <http://www.ncbi.nlm.nih.gov/>).

## Structure of the ScMAB:

#	Nucleic Acid	Function
1-66		pelB signal sequence
67-468		VH anti-Tumor
469-483		Linker L1
484-819		VL anti-FcγRI
820-864		Peptide Linker P
865-1224		VH anti-FcγRI
1225-1239		Linker L2
1240-1561		VL anti-Tumor
1562-1564		Stop codon

ATGAAATACCTATTGCCTACGGCAGCCGCTGGATTGTTATTACTCGCGGC  
CCAGCCGGCCATGGCCcaggtgcagctggtgcagctctggggctgaggtga  
agaagcctggggcctcagtgaagggttctcgaaggcatctggatacacc  
ttcaccagctactatatgcactgggtcgaacaggcccctggacaagggct  
tgagtggatgggaataatcaaccctagtggtagcacaagctacgcac  
agaagttccagggcagagtcaccatgaccaggacacgtccacgagcaca  
gtctacatggagctgagcagcctgagatctgaggacacggccgtgtatta  
ctgtgctagagaggatgggcctacagctatggccacaagggccgaccta  
cgacaacatactactactactacggtatggacgtctggggccaagggacc  
acggtcaccgtctcctcaGGCGGTGGCGGATCGgacatccagctgaccca  
gagcccaagcagcctgagcgcagcgtgggtgacagagtgaccatcacct  
gtaagtccagtc aaagtgttttatacagttcaaatacagaagaactacttg  
gcctggtaccagcagaagccaggttaaggctccaaagctgctgatctactg  
ggcatccactagggaaatctggtgtgccaagcagattcagcggtagcggta  
gcggtaccgacttcaccttcacatcagcagcctccagccagaggacatc  
gccacctactactgccatcaatacctctcctcgtggacgttcggccaagg  
gaccaaggtggaaatcaaaGGAGGCGGTGGCAGCGGTGGGCGCGCCTCGG  
GCGGAGGTGGCTCAcaggtccaactgcaggagagcgggtccaggtcttg  
agacctagccagaccctgagcctgacctgcaccgtgtctggcttcatttt  
cagcgacaattacatgtattgggtgagacagccacctggacgaggtcttg  
agtggattggaaccattagtgtggtggttagttacacctactatcctgac



agtgtgaaggggagagtgacaatgctgagagacaccagcaagaaccagtt  
 cagcctgagactcagcagcgtgacagccgccgacaccgcggtctattatt  
 gtgcaagaggctactataggtacgagggggctatggactactggggccaa  
 gggtccttggtcaccgtctcctcaGGAGGCGGGGGTTCGgatatcgagct  
 cccaccctcggtgtcagtggtccctaggacagatggccaggatcacctgct  
 ctggagaagcattgccaaaaaatatgcttattggtaccagcagaagcca  
 ggccagttccctgtgctggtgatataaaagacagcgagagggcctcagg  
 gatccctgagcgattctctggtccagctcagggacaatagtcacattga  
 ccatcagtgaggtccaggcagaagacgaggctgactattactgtctatca  
 gcagacagcagtggtacttatggcgggtggttcggcggaggggaccaagct  
 gaccgtcctaTAA

1/1	31/11
61/21	91/31

ATG AAA TAC CTA TTG CCT ACG GCA GCC GCT GGA TTG TTA TTA CTC GCG  
 GCC CAG CCG GCC ATG GCC cag gtg cag ctg gtg cag tct ggg gct gag  
 gtg aag aag cct ggg gcc tca gtg

M	K	Y	L	L	P	T	A	A	A	G	L	L	L	L	A	A
Q	P	A	M	A	Q	V	Q	L	V	Q	S	G	A	E	V	K
K	P	G	A	S	V											

121/41	151/51
181/61	211/71

aag gtt tcc tgc aag gca tct gga tac acc ttc acc agc tac tat atg  
 cac tgg gtc gaa cag gcc cct gga caa ggg ctt gag tgg atg gga ata  
 atc aac cct agt ggt ggt agc aca

K	V	S	C	K	A	S	G	Y	T	F	T	S	Y	Y	M	H
W	V	E	Q	A	P	G	Q	G	L	E	W	M	G	I	I	N
P	S	G	G	S	T											

241/81	271/91
301/101	331/111

agc tac gca cag aag ttc cag ggc aga gtc acc atg acc agg gac acg  
 tcc acg agc aca gtc tac atg gag ctg agc agc ctg aga tct gag gac  
 acg gcc gtg tat tac tgt gct aga

S	Y	A	Q	K	F	Q	G	R	V	T	M	T	R	D	T	S
T	S	T	V	Y	M	E	L	S	S	L	R	S	E	D	T	A
V	Y	Y	C	A	R											

361/121	391/131
421/141	451/151

gag gat ggg cct aca gct atg gcc aca agg gcc gac cta acg aca aca  
tac tac tac tac tac ggt atg gac gtc tgg ggc caa ggg acc acg gtc  
acc gtc tcc tca GGC GGT GGC GGA  
  
E D G P T A M A T R A D L T T T Y  
Y Y Y Y G M D V W G Q G T T V T V  
S S G G G G  
  
481/161 511/171  
541/181 571/191  
  
TCG gac atc cag ctg acc cag agc cca agc agc ctg agc gcc agc gtg  
ggt gac aga gtg acc atc acc tgt aag tcc agt caa agt gtt tta tac  
agt tca aat cag aag aac tac ttg  
  
S D I Q L T Q S P S S L S A S V G  
D R V T I T C K S S Q S V L Y S S  
N Q K N Y L  
  
601/201 631/211  
661/221 691/231  
  
gcc tgg tac cag cag aag cca ggt aag gct cca aag ctg ctg atc tac  
tgg gca tcc act agg gaa tct ggt gtg cca agc aga ttc agc ggt agc  
ggt agc ggt acc gac ttc acc ttc  
  
A W Y Q Q K P G K A P K L L I Y W  
A S T R E S G V P S R F S G S G S  
G T D F T F  
  
721/241 751/251  
781/261 811/271  
  
acc atc agc agc ctc cag cca gag gac atc gcc acc tac tac tgc cat  
caa tac ctc tcc tcg tgg acg ttc ggc caa ggg acc aag gtg gaa atc  
aaa GGA GGC GGT GGC AGC GGT GGG  
  
T I S S L Q P E D I A T Y Y C H Q  
Y L S S W T F G Q G T K V E I K G  
G G G S G G  
  
841/281 871/291  
901/301 931/311  
  
CGC GCC TCG GGC GGA GGT GGC TCA cag gtc caa ctg cag gag agc ggt  
cca ggt ctt gtg aga cct agc cag acc ctg agc ctg acc tgc acc gtg  
tct ggc ttc att ttc agc gac aat  
  
R A S G G G G S Q V Q L Q E S G P  
G L V R P S Q T L S L T C T V S G  
F I F S D N  
  
961/321 991/331  
1021/341 1051/351  
  
tac atg tat tgg gtg aga cag cca cct gga cga ggt ctt gag tgg att  
gga acc att agt gat ggt ggt agt tac acc tac tat cct gac agt gtg  
aag ggg aga gtg aca atg ctg aga

Y M Y W V R Q P P G R G L E W I G  
T I S D G G S Y T Y Y P D S V K G  
R V T M L R

1081/361

1111/371

1141/381

1171/391

gac acc agc aag aac cag ttc agc ctg aga ctc agc agc gtg aca gcc  
gcc gac acc gcg gtc tat tat tgt gca aga ggc tac tat agg tac gag  
ggg gct atg gac tac tgg ggc caa

D T S K N Q F S L R L S S V T A A  
D T A V Y Y C A R G Y Y R Y E G A  
M D Y W G Q

1201/401

1231/411

1261/421

1291/431

ggg tcc ttg gtc acc gtc tcc tca GGA GGC GGG GGT TCG gat atc gag  
ctc cca ccc tcg gtg tca gtg tcc cta gga cag atg gcc agg atc acc  
tgc tct gga gaa gca ttg cca aaa

G S L V T V S S G G G G S D I E L  
P P S V S V S L G Q M A R I T C S  
G E A L P K

1321/441

1351/451

1381/461

1411/471

aaa tat gct tat tgg tac cag cag aag cca ggc cag ttc cct gtg ctg  
gtg ata tat aaa gac agc gag agg gcc tca ggg atc cct gag cga ttc  
tct ggc tcc agc tca ggg aca ata

K Y A Y W Y Q Q K P G Q F P V L V  
I Y K D S E R A S G I P E R F S G  
S S S G T I

1441/481

1471/491

1501/501

1531/511

gtc aca ttg acc atc agt gga gtc cag gca gaa gac gag gct gac tat  
tac tgt cta tca gca gac agc agt ggt act tat ggc ggg tgg ttc ggc  
gga ggg acc aag ctg acc gtc cta

V T L T I S G V Q A E D E A D Y Y  
C L S A D S S G T Y G G W F G G G  
T K L T V L

1561/521

TAA

\*

3. ScMAB anti-Tumor x anti-herpes virus glycoprotein B (based on sequences with the accession number Seq. S77598.1 / S77599.1 and L37310.1 / L37301.1) for the retargeting of herpes virus to tumor cells (anti-herpes glycoprotein B anti-body specific for herpes viral coat proteins, Ohlin,M. et al., Mol. Immunol. 33 (1), 47-56 (1996), sequence database: National Center for Biological Resources; and anti-tumor Antibody specific for ovarina, cerix, breast and colon carcinoma, i.e. specific for membrane structures of tumor cells, Chen,P.F., et al., Hum. Antibodies Hybridomas 5 (3-4), 131-142 (1994), Sequence database: National Center for Biological Resources, (NCBI, accessible through <http://www.ncbi.nlm.nih.gov/>).

#### Structure of the ScMAB:

#	Nucleic Acid	Function
1-66		pelB signal sequence
67-468		VH anti-Tumor
469-483		Linker L1
484-804		VL anti-herpes virus glycoprotein B
805-849		Peptide Linker P
850-1218		VH anti- herpes virus glycoprotein B
1219-1233		Linker L2
1234-1554		VL anti-Tumor
1555-1557		Stop codon

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ATGAAATACCTATTGCCTACGGCAGCCGCTGGATTGTTATTACTCGCGGC
CCAGCCGGCCATGGCCcaggtgcagctggtgcagtctggggctgaggtga
agaagcctggggcctcagtgaagggttctcctgcaaggcatctggatacacc
ttcaccagctactatatgcactgggtcgaacaggcccctggacaagggct
tgagtggatgggaataatcaaccctagtggtggtagcacaagctacgcac
agaagttccagggcagagtcaccatgaccagggaacagtcacagagcaca
gtctacatggagctgagcagcctgagatctgaggacacggccgtgtatta
ctgtgctagagaggatgggcctacagctatggccacaagggccgacctaa
cgacaacatactactactactacggtatggacgtctggggccaagggacc
acggtcaccgtctcctcaGGCGGTGGCGGATCGgaaattgtgttgacgca
gtctccagccaccctgtctttgtctccaggggaaagagccaccctctcct

```

gcagggccagtcagagtggtggcagctccttagcctggtaccaacagaaa  
cctggccaggctcccaggctcctcgtctatgatacatccaacagggccac  
tggcatcccagccaggttcaatggcagtggtctgggacagacttcactc  
tcaccatcagcagcctagagcctgaagattttgcagattattactgtcaa  
cagcgaagcgagtggtcctctcactttcggcggagggaccaaggtggagat  
caaaGGAGGCGGTGGCAGCGGTGGGCGCGCCTCGGGCGGAGGTGGCTCAc  
agctgcagctggtggagctctgggggagggcgtggtccagcctgggaggtcc  
ttgagactctcctgtgcagcctctgggttcattttcagtgaagtatgatat  
gcactgggtccgccaggctccaggcaaggggctgcagtggtggcagtta  
tatcagttgatggaagtataaacactacgcagactccgtgaagggccga  
ttcaccatctccagagacaattcccagaacatgttgtttctacaaatgga  
cagcctgagaggtgacgacacggctgtttattattgtgcgagagatggaa  
aaagtttgaatggttattccggcttgattgactactggggccagggatcc  
ttagtcaccgtctcctcaGGAGGCGGGGGTTCGgatatcgagctcccacc  
ctcgggtgtcagtgctccttaggacagatggccaggatcacctgctctggag  
aagcattgccaaaaaatatgcttattggtaccagcagaagccaggccag  
ttcctgtgctggtgatataaaagacagcgagagggcctcagggatccc  
tgagcgattctctggctccagctcagggacaatagtcacattgaccatca  
gtggagtcaggcagaagacgaggctgactattactgtctatcagcagac  
agcagtggtacttatggcgggtggttcggcggagggaccaagctgaccgt  
cctaTAA

1/1  
61/21

31/11  
91/31

ATG AAA TAC CTA TTG CCT ACG GCA GCC GCT GGA TTG TTA TTA CTC GCG  
GCC CAG CCG GCC ATG GCC cag gtg cag ctg gtg cag tct ggg gct gag  
gtg aag aag cct ggg gcc tca gtg

M	K	Y	L	L	P	T	A	A	A	G	L	L	L	L	A	A
Q	P	A	M	A	Q	V	Q	L	V	Q	S	G	A	E	V	K
K	P	G	A	S	V											

121/41  
181/61

151/51  
211/71

aag gtt tcc tgc aag gca tct gga tac acc ttc acc agc tac tat atg  
cac tgg gtc gaa cag gcc cct gga caa ggg ctt gag tgg atg gga ata  
atc aac cct agt ggt ggt agc aca  
K V S C K A S G Y T F T S Y Y M H  
W V E Q A P G Q G L E W M G I I N  
P S G G S T  
241/81 271/91  
301/101 331/111  
agc tac gca cag aag ttc cag ggc aga gtc acc atg acc agg gac acg  
tcc acg agc aca gtc tac atg gag ctg agc agc ctg aga tct gag gac  
acg gcc gtg tat tac tgt gct aga  
S Y A Q K F Q G R V T M T R D T S  
T S T V Y M E L S S L R S E D T A  
V Y Y C A R  
361/121 391/131  
421/141 451/151  
gag gat ggg cct aca gct atg gcc aca agg gcc gac cta acg aca aca  
tac tac tac tac tac ggt atg gac gtc tgg ggc caa ggg acc acg gtc  
acc gtc tcc tca GGC GGT GGC GGA  
E D G P T A M A T R A D L T T T Y  
Y Y Y Y G M D V W G Q G T T V T V  
S S G G G G  
481/161 511/171  
541/181 571/191  
TCG gaa att gtg ttg acg cag tct cca gcc acc ctg tct ttg tct cca  
ggg gaa aga gcc acc ctc tcc tgc agg gcc agt cag agt gtt ggc agc  
tcc tta gcc tgg tac caa cag aaa  
S E I V L T Q S P A T L S L S P G  
E R A T L S C R A S Q S V G S S L  
A W Y Q Q K  
601/201 631/211  
661/221 691/231  
cct ggc cag gct ccc agg ctc ctc gtc tat gat aca tcc aac agg gcc  
act ggc atc cca gcc agg ttc aat ggc agt ggg tct ggg aca gac ttc  
act ctc acc atc agc agc cta gag  
P G Q A P R L L V Y D T S N R A T  
G I P A R F N G S G S G T D F T L  
T I S S L E  
721/241 751/251  
781/261 811/271  
cct gaa gat ttt gca gat tat tac tgt caa cag cga agc gag tgg cct  
ctc act ttc ggc gga ggg acc aag gtg gag atc aaa GGA GGC GGT GGC  
AGC GGT GGG CGC GCC TCG GGC GGA

P E D F A D Y Y C Q Q R S E W P L  
T F G G G T K V E I K G G G G S G  
G R A S G G

841/281  
901/301

871/291  
931/311

GGT GGC TCA cag ctg cag ctg gtg gag tct ggg gga ggc gtg gtc cag  
cct ggg agg tcc ttg aga ctc tcc tgt gca gcc tct ggg ttc att ttc  
agt gag tat gat atg cac tgg gtc

G G S Q L Q L V E S G G G V V Q P  
G R S L R L S C A A S G F I F S E  
Y D M H W V

961/321  
1021/341

991/331  
1051/351

cgc cag gct cca ggc aag ggg ctg cag tgg gtg gca gtt ata tca gtt  
gat gga agt gat aaa cac tac gca gac tcc gtg aag ggc cga ttc acc  
atc tcc aga gac aat tcc cag aac

R Q A P G K G L Q W V A V I S V D  
G S D K H Y A D S V K G R F T I S  
R D N S Q N

1081/361  
1141/381

1111/371  
1171/391

atg ttg ttt cta caa atg gac agc ctg aga ggt gac gac acg gct gtt  
tat tat tgt gcg aga gat gga aaa agt ttg aat ggt tat tcc ggc ttg  
att gac tac tgg ggc cag gga tcc

M L F L Q M D S L R G D D T A V Y  
Y C A R D G K S L N G Y S G L I D  
Y W G Q G S

1201/401  
1261/421

1231/411  
1291/431

tta gtc acc gtc tcc tca GGA GGC GGG GGT TCG gat atc gag ctc cca  
ccc tcg gtg tca gtg tcc cta gga cag atg gcc agg atc acc tgc tct  
gga gaa gca ttg cca aaa aaa tat

L V T V S S G G G G S D I E L P P  
S V S V S L G Q M A R I T C S G E  
A L P K K Y

1321/441  
1381/461

1351/451  
1411/471

gct tat tgg tac cag cag aag cca ggc cag ttc cct gtg ctg gtg ata  
tat aaa gac agc gag agg gcc tca ggg atc cct gag cga ttc tct ggc  
tcc agc tca ggg aca ata gtc aca

A Y W Y Q Q K P G Q F P V L V I Y  
K D S E R A S G I P E R F S G S S  
S G T I V T

1441/481

1471/491

1501/501

1531/511

ttg acc atc agt gga gtc cag gca gaa gac gag gct gac tat tac tgt  
cta tca gca gac agc agt ggt act tat ggc ggg tgg ttc ggc gga ggg  
acc aag ctg acc gtc cta TAA

L	T	I	S	G	V	Q	A	E	D	E	A	D	Y	Y	C	L
S	A	D	S	S	G	T	Y	G	G	W	F	G	G	G	T	K
L	T	V	L	*												